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APPLICATION NUMBER: *60/434,952*
FILING DATE: *December 19, 2002*

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PTO/SB/16 (08-00)
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JC525 U.S. PTO 12/19/02

JC846 U.S. PTO 60/434952 12/19/02

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. § 1.53(c).

Express Mail Label No.: EL827179211US	Atty Docket Number: H053912.0015US0	Type a plus sign (+) inside this box → +

INVENTOR(S)

Given Name (first and middle)	Last Name	Residence (City and either State or Foreign Country)
Maria Gabriella	Brasca	Cusago, Italy
Raffaella	Amici	Piacenza, Italy
Daniele	Fancelli	Milano, Italy
Marcella	Nesi	Saronno, Italy

TITLE OF THE INVENTION (280 characters max)

SUBSTITUTED PYRROLO- AND PYRIDINO-PYRAZOLES AS KINASE INHIBITORS

CORRESPONDENCE ADDRESS

Customer Number: 001200
AKIN, GUMP, STRAUSS, HAUER & FELD, L.L.P.
711 LOUISIANA, SUITE 1900
HOUSTON

Place Customer Number Barcode Label here

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PATENT_TRADEMARK OFFICE

State	TEXAS	Zip Code	77002	Country	UNITED STATES

ENCLOSED APPLICATION PARTS (check all that apply)

■	Specification	Number of Pages	65	☐	CD(s), Number _____
■	Drawing(s)	Number of Pages	0	☐	Other (specify) _____
☐	Application Data Sheet. See 37CFR 176				

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)

		FILING FEE AMOUNT ($)
☐	Applicant claims small entity status. See 37 CFR 1.27.	$ 160.00
■	A check or money order is enclosed to cover the filing fees	
☐	The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account No. 16-2435	
☐	Payment by credit card. Form PTO-2038 is attached.	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

■ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ Date: 12/19/02

Dwayne L. Mason, Reg. No. 38,959

■ Additional inventors are being named on separately numbered sheets attached hereto.

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLIATION COVER SHEET
Additional Page

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Docket Number:	H053912.0015US0	Type a plus sign (+) inside this box →	+

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle[if any])	Family or Surname	Residence (City and either State or Foreign Country)
Paolo	Orsini	Gallarate, Italy
Fabrizio	Orzi	Milano, Italy
Patrick	Roussel	Nerviano, Italy
Anna	Vulpetti	Brugherio, Italy
Paolo	Pevarello	Pavia, Italy

Number 2 of 2

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PTO/SB/17 (03/01)

FEE TRANSMITTAL for FY 2002

Patent fees are subject to annual revision

Complete if Known	
Application Number	
Filing Date	December 18, 2002
First Named Inventor	Maria Gabriella Brasca
Examiner Name	
Group / Art Unit	
Attorney Docket No.	H053912.0015US0

Total Amount of Payment $160.00

METHOD OF PAYMENT (check one)

1. X The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
   Deposit Account No.: 16-2435
   Deposit Account Name: Akin, Gump, Strauss, Hauer & Feld, L.L.P.
   X Charge any additional Fee Required Under 37 CFR §§ 1.16 & 1.17
   ___ Applicant claims small entity status. See 37 CFR 1.27

2. X Payment Enclosed: X Check ___ Money Order ___ Credit Card ___ Other

FEE CALCULATION

1. Basic Filing Fee

Large Fee Code	Large Entity Fee ($)	Small Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	$740	201	$370	Utility Filing Fee	$
106	$330	206	$165	Design Filing Fee	$
107	$510	207	$255	Plant Filing Fee	$
108	$740	208	$370	Reissue Filing Fee	$
114	$160	214	$ 80	Provisional Filing Fee	$ 160
				Subtotal (1)	$160.00

2. Extra Claim Fees

Claims		Extra		Fee (below)		Fee Paid
Total	-20** =		x	$	=	$
Indep.	- 3** =		x	$	=	$
Multiple Dependent						$

**or number previously paid, if greater.

For Reissues, see below

Large Entity Fee ($)	Small Entity Fee ($)	Fee Description
$ 18	$ 9	Claims in excess of 20
$ 84	$ 42	Independent claims in excess of 3
$ 280	$ 140	Multiple dependent claim, if not paid
$ 84	$ 42	**Reissue independent claims over original patent
$ 18	$ 9	**Reissue claims in excess of 20 and over original patent
		Subtotal (2) $0.00

FEE CALCULATION (continued)

3. Additional Fees

Large Fee Code	Large Entity Fee ($)	Small Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	$ 130	205	$ 65	Surcharge - late fee or oath	$
127	$ 50	227	$ 25	Surcharge - late provisional filing fee or cover sheet	$
147	$2,520	147	$2,520	Request for Reexamination	$
112	$920*	112	$920*	Requesting publication of SIR prior to Examiner action	$
113	$1840*	113	$1840*	Requesting publication of SIR after Examiner action	$
115	$ 110	215	$ 55	Extension for reply within first month	$
116	$ 400	216	$ 200	Extension for reply within second month	$
117	$ 920	217	$ 460	Extension for reply within third month	$
118	$1,440	218	$ 720	Extension for reply within fourth month	$
128	$1,960	228	$ 980	Extension for reply within fifth month	$
119	$ 320	219	$ 160	Notice of Appeal	$
120	$ 320	220	$ 160	Filing a brief in support of an appeal	$
121	$ 280	221	$ 140	Request for oral hearing	$
140	$ 110	240	$ 55	Petition to revive - unavoidable	$
141	$1,280	241	$ 640	Petition to revive - unintentional	$
142	$1,280	242	$ 640	Utility issue fee (or reissue)	$
143	$ 460	243	$ 230	Design issue fee	$
144	$ 620	244	$ 310	Plant issue fee	$
122	$ 130	122	$ 130	Petitions to the Commissioner	$
123	$ 50	123	$ 50	Petitions related to provisional applications	$
126	$ 180	126	$ 180	Submission of Information Disclosure Statement	$
581	$ 40	581	$ 40	Recording each patent assignment per property (times number of properties)	$
146	$ 740	246	$ 370	Filing a submission after final rejection (37 CFR 1.129(a))	$
179	$ 740	279	$ 370	Request for Continued Examination (RCE)	$
169	$ 900	169	$ 900	Request for expedited examination of a design application	$

Other fee (specify): _____ $

Other fee (specify): _____ $

* Reduced by Basic Filing Fee Paid

Subtotal (3) $0.00

Submitted by

Date: 12/19/02

Dwayne L. Mason, Reg. No. 38,959

053912.0015 HOUSTON 260186 v1 (01161 MP/lc)

U.S. EXPRESS MAIL AIRBILL NO. EL827179211US

TITLE OF THE INVENTION

SUBSTITUTED PYRROLO- AND PYRIDINO-PYRAZOLES AS KINASE INHIBITORS

SPECIFICATION

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to pyrrolo- and pyridino-pyrazole derivatives, to a process for their preparation, to pharmaceutical compositions comprising them, and to their use as therapeutic agents, particularly in the treatment of cancer and cell proliferation disorders.

Discussion of the Background

Several cytotoxic drugs such as, e.g., fluorouracil (5-FU), doxorubicin and camptothecins, damage DNA or affect cellular metabolic pathways and thus cause, in many cases, an indirect block of the cell cycle. Therefore, by producing an irreversible damage to both normal and tumor cells, these agents result in a significant toxicity and side-effects. In this respect, compounds capable of functioning as highly specific antitumor agents by selectively leading to tumor cell arrest and apoptosis, with comparable efficacy but reduced toxicity than the currently available drugs, are desirable.

It is well known that progression through the cell cycle is governed by a series of checkpoint controls, otherwise referred to as restriction points, which are regulated by a family of enzymes known as the Cyclin-dependent kinases (Cdk). In turn, the Cdks themselves are regulated at many levels such as, for instance, binding to cyclins.

The coordinated activation and inactivation of different Cdk/Cyclin complexes is necessary for normal progression through the cell cycle. Both the critical G1-S and G2-M transitions are controlled by the activation of different Cdk/Cyclin activities. In G1, both Cdk4/Cyclin D and Cdk2/Cyclin E are thought to mediate the onset of S-phase. Progression through S-phase requires the activity of Cdk2/Cyclin A whereas the activation of Cdc2/Cyclin A (Cdk1) and Cdc2/cyclin B are required for the onset of mitosis. For a general reference to cyclins and cyclin-dependent kinases see, for instance, Kevin R. Webster et al, in Exp. Opin. Invest. Drugs, 1998, Vol. 7(6), 865-887.

Checkpoint controls are defective in tumor cells due, in part, to disregulation of cdk activity. For example, altered expression of cyclin E and cdks has been observed in tumor

cells, and deletion of the cdk inhibitor p27 KIP gene in mice has been shown to result in a higher incidence of cancer.

Increasing evidence supports the idea that the cdks are rate-limiting enzymes in cell cycle progression and, as such, represent molecular targets for therapeutic intervention. In particular, the direct inhibition of cdk/cyclin kinase activity should be helpful in restricting the unregulated proliferation of a tumor cell.

SUMMARY OF THE INVENTION

It is an object of the invention to provide compounds which are useful in treating cell proliferative disorders caused by and/or associated with an altered cell cycle dependent kinase activity. It is another object to provide compounds which have cdk/cyclin kinase inhibitory activity.

The present inventors have now discovered that certain pyrazoles are endowed with cdk/cyclin kinase inhibitory activity and are thus useful in therapy as antitumor agents and lack, in terms of both toxicity and side effects, the aforementioned drawbacks associated with currently available antitumor drugs.

More specifically, the pyrazoles of the invention are useful in the treatment of a variety of cancers including, but not limited to: carcinoma such as bladder, breast, colon, kidney, liver, lung, including small cell lung cancer, esophagus, gall-bladder, ovary, pancreas, stomach, cervix, thyroid, prostate, and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage including leukaemia, acute lymphocitic leukaemia, acute lymphoblastic leukaemia, B-cell lymphoma, T-cell-lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias, myelodysplastic syndrome and promyelocytic leukaemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; tumors of the central and peripheral nervous system, including astrocytoma neuroblastoma, glioma and schwannomas; other tumors, including melanoma, seminoma, teratocarcinoma, osteosarcoma, xeroderma pigmentosum, keratoxanthoma, thyroid follicular cancer and Kaposi's sarcoma.

Due to the key role of cdks in the regulation of cellular proliferation, these pyrazole derivatives are also useful in the treatment of a variety of cell proliferative disorders such as, for example, benign prostate hyperplasia, familial adenomatosis polyposis, neurofibromatosis, psoriasis, vascular smooth cell proliferation associated with

atherosclerosis, pulmonary fibrosis, arthritis, glomerulonephritis and post-surgical stenosis and restenosis.

The compounds of the invention may be useful in treatment of Alzheimer's disease, as suggested by the fact that cdk5 is involved in the phosphorylation of tau protein (J. Biochem. 117, 741-749, 1995).

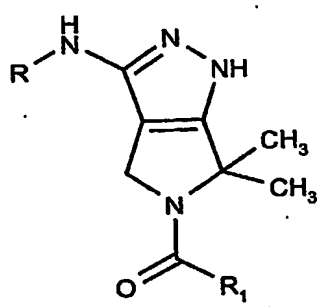
The compounds of this invention, as modulators of apoptosis, may also be useful in the treatment of cancer, viral infections, prevention of AIDS development in HIV-infected individuals, autoimmune diseases and neurodegenerative disorders.

The compounds of this invention may be useful in inhibiting tumor angiogenesis and metastasis, as well as in the treatment of organ transplant rejection and host versus graft disease.

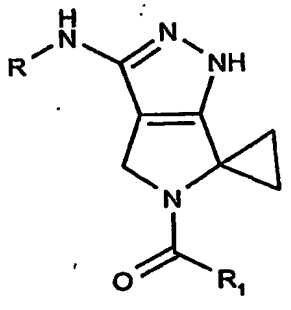
The compounds of the invention may also act as inhibitor of other protein kinases, e.g., protein kinase C in different isoforms, Met, PAK-4, PAK-5, ZC-1, STLK-2, DDR-2, Aurora 1, Aurora 2, Bub-1, PLK, Chk1, Chk2, HER2, raf1, MEK1, MAPK, EGF-R, PDGF-R, FGF-R, IGF-R, PI3K, weel kinase, Src, Abl, Akt, MAPK, ILK, MK-2, IKK-2, Cdc7, Nek, and thus be effective in the treatment of diseases associated with other protein kinases.

The compounds of the invention are also useful in the treatment and prevention of radiotherapy-induced or chemotherapy-induced alopecia.

Accordingly, the present invention provides a method for treating cell proliferative disorders caused by and/or associated with an altered cell cycle dependent kinase activity, by administering to a mammal in need thereof an effective amount of a pyrazole derivative represented by formula (Ia) or (Ib)



(Ia)



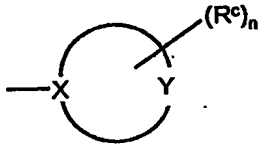
(Ib)

wherein

R is a group -COR^a, -CONHR^a or -CONR^{a}R^b wherein R^a and R^b are, each independently, hydrogen or an optionally substituted group selected from straight or branched

C_1-C_6 alkyl, C_3-C_6 cycloalkyl, aryl, arylalkyl, heterocyclyl or heterocyclylalkyl or; together with the nitrogen atom to which they are bonded, R^a and R^b may form an optionally substituted 5 or 6 membered heterocycle optionally containing one additional heteroatom or heteroatomic group selected among N, NH, O or S;

R_1 is tert-butyl or a group of formula (II)



(II)

representing a 5 to 7 membered heterocyclic ring wherein X, directly linked to the rest of the molecule, represents a carbon or nitrogen atom; Y is a carbon, nitrogen, oxygen or sulfur atom or it is an NH group, provided that at least one of X and Y is other than a carbon atom; R^c is, independently from each other and in any one of the free positions of the heterocyclic ring of formula (II), an optionally substituted group selected from straight or branched C_1-C_6 alkyl, C_3-C_6 cycloalkyl, aryl, arylalkyl, heterocyclyl, heterocyclylalkyl, amino, aminocarbonyl, carboxy, oxo (=O), alkoxycarbonyl, alkylcarbonyl or arylcarbonyl; and n is 0 or an integer from 1 to 4;

or a pharmaceutically acceptable salt thereof.

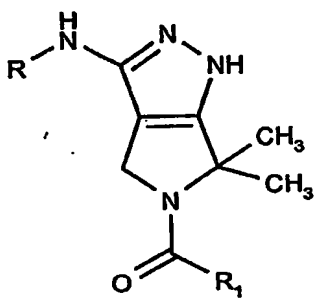
In a preferred embodiment of the method described above, the cell proliferative disorder is selected from the group consisting of cancer, Alzheimer's disease, viral infections, auto-immune diseases and neurodegenerative disorders.

Specific types of cancer that may be treated include carcinoma, squamous cell carcinoma, hematopoietic tumors of myeloid or lymphoid lineage, tumors of mesenchymal origin, tumors of the central and peripheral nervous system, melanoma, seminoma, teratocarcinoma, osteosarcoma, xeroderma pigmentosum, keratoxanthoma, thyroid follicular cancer, and Kaposi's sarcoma.

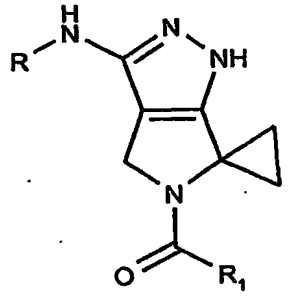
In another preferred embodiment of the method described above, the cell proliferative disorder is selected from the group consisting of benign prostate hyperplasia, familial adenomatosis polyposis, neuro-fibromatosis, psoriasis, vascular smooth cell proliferation associated with atherosclerosis, pulmonary fibrosis, arthritis, glomerulonephritis and post-surgical stenosis and restenosis. In addition, the inventive method provides tumor angiogenesis and metastasis inhibition as well as treatment of organ transplant rejection and host versus graft disease. The inventive method may also provide cell cycle inhibition or cdk/cyclin dependent inhibition.

In addition to the above, the method object of the present invention provides treatment and prevention of radiotherapy-induced or chemotherapy-induced alopecia.

The present invention also provides a pyrazole derivative represented by formula (Ia) or (Ib)



(Ia)

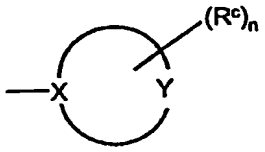


(Ib)

wherein

R is a group $-COR^a$, $-CONHR^a$ or $-CONR^aR^b$ wherein R^a and R^b are, each independently, hydrogen or an optionally substituted group selected from straight or branched C_1-C_6 alkyl, C_3-C_6 cycloalkyl, aryl, arylalkyl, heterocyclyl or heterocyclylalkyl or; together with the nitrogen atom to which they are bonded, R^a and R^b may form an optionally substituted 5 or 6 membered heterocycle optionally containing one additional heteroatom or heteroatomic group selected among N, NH, O or S;

R_1 is tert-butyl or a group of formula (II)



(II)

representing a 5 to 7 membered heterocyclic ring wherein X, directly linked to the rest of the molecule, represents a carbon or nitrogen atom; Y is a carbon, nitrogen, oxygen or sulfur atom or it is an NH group, provided that at least one of X and Y is other than a carbon atom; R^c is, independently from each other and in any one of the free positions of the heterocyclic ring of formula (II), an optionally substituted group selected from straight or branched C_1-C_6 alkyl, C_3-C_6 cycloalkyl, aryl, arylalkyl, heterocyclyl, heterocyclylalkyl, amino, aminocarbonyl, carboxy, oxo (=O), alkoxycarbonyl, alkylcarbonyl or arylcarbonyl; and n is 0 or an integer from 1 to 4;

or a pharmaceutically acceptable salt thereof.

The present invention also includes methods for the synthesis of the pyrazole derivatives represented by formulae (Ia) or (Ib) that, unless otherwise provided, may be conveniently grouped and defined as compounds of formula (I). Pharmaceutical

compositions comprising the pyrazoles of formula (I) are also included in the present invention.

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

Several heterocyclic compounds are known in the art as protein kinase inhibitors. As an example, 2-carboxamido-pyrazoles and 2-ureido-pyrazoles, and derivatives thereof, have been disclosed as protein kinase inhibitors in the international patent applications WO 01/12189, WO 01/12188, WO 02/48114 and WO 02/70515, all in the name of the applicant itself.

Fused bicyclic compounds comprising pyrazole moieties and possessing kinase inhibitory activity have been also disclosed in WO 00/69846 and WO 02/12242 as well as in copending and still unpublished PCT/EP02/10534 (claiming priority from US patent application No. 09/962162, filed in September 26, 2001) and US patent application 60/381092 (filed in May 17, 2002), all in the name of the applicant itself.

The compounds object of the present invention fall within the scope of the general formula of the aforementioned WO 02/12242, herewith incorporated by reference, but are not specifically exemplified therein.

The compounds of formula (Ia) or (Ib) of the invention, hereinafter referred to as compounds of formula (I), may have asymmetric carbon atoms and may therefore exist as individual optical isomers, as racemic admixtures or as any other admixture comprising a majority of one of the two optical isomers, which are all to be intended as within the scope of the present invention.

Likewise, the use as an antitumor agent of all the possible isomers and their admixtures and of both the metabolites and the pharmaceutically acceptable bio-precursors (otherwise referred to as pro-drugs) of the compounds of formula (I) are also within the scope of the present invention.

In the present description, unless otherwise specified, with the term straight or branched C_1-C_6 alkyl we intend any of the groups such as, for instance, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, sec-butyl, n-pentyl, n-hexyl, and the like.

With the term C_3-C_6 cycloalkyl we intend, unless otherwise provided, a cycloaliphatic ring such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

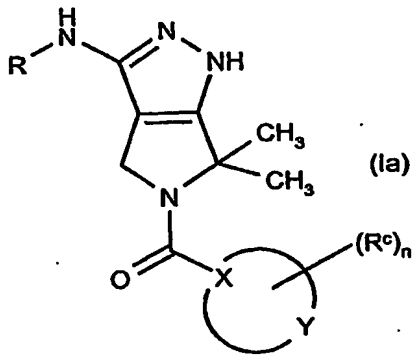
The term aryl includes carbocyclic or heterocyclic hydrocarbons with from 1 to 2 ring moieties, either fused or linked to each other by single bonds, wherein at least one of the rings is aromatic; if present, any aromatic heterocyclic hydrocarbon also referred to as heteroaryl group, comprises a 5 to 6 membered ring with from 1 to 3 heteroatoms or heteroatomic groups selected among N, NH, O or S.

Examples of aryl groups according to the invention are, for instance, phenyl, biphenyl, α- or β-naphthyl, dihydronaphthyl, thienyl, benzothienyl, furyl, benzofuranyl, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, isoindolyl, purinyl, quinolyl, isoquinolyl, dihydroquinolinyl, quinoxalinyl, benzodioxolyl, indanyl, indenyl, triazolyl, and the like.

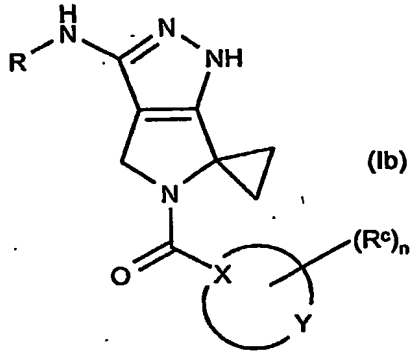
Unless otherwise specified, the term heterocyclyl includes 5 to 6 membered saturated, partly unsaturated or fully unsaturated heterocycles with from 1 to 3 heteroatoms or heteroatomic groups selected among N, NH, O or S.

Apart from the fully unsaturated heterocycles, previously referred to as aromatic heterocycles and encompassed by the term aryl, examples of saturated or partly unsaturated heterocycles according to the invention are, for instance, pyran, pyrrolidine, pyrroline, imidazoline, imidazolidine, pyrazolidine, pyrazoline, thiazoline, thiazolidine, dihydrofuran, tetrahydrofuran, 1,3-dioxolane, piperidine, piperazine, morpholine and the like.

When referring to the compounds of the invention wherein R_I is a group of formula (II), the 5 to 7 membered heterocyclic ring is directly linked to the rest of the molecule through the X atom, as follows:



(Ia)



(Ib)

Examples of 5 to 7 membered heterocycles include any 5 to 6 membered heterocycle among those already reported and, additionally, 7 membered heterocycles such as, for instance, azepine, diazepine, oxazepine and the like.

Any R^c, if present, is at any one of the free positions of the heterocyclic ring of formula (II) by replacement of a hydrogen atom.

According to the present invention and unless otherwise provided, any of the above R^a, R^b and R^c groups may be optionally substituted, in any of their free positions, by one or more groups, for instance 1 to 6 groups, independently selected from: halogen, nitro, oxo groups (=O), cyano, alkyl, perfluorinated alkyl, perfluorinated alkoxy, alkenyl, alkynyl, hydroxyalkyl, aryl, arylalkyl, heterocyclyl, cycloalkyl, hydroxy, alkoxy, aryloxy, heterocyclyloxy, methylenedioxy, alkylcarbonyloxy, arylcarbonyloxy, cycloalkenyloxy, alkylideneaminooxy, carboxy, alkoxycarbonyl, aryloxycarbonyl, cycloalkyloxycarbonyl, amino, ureido, alkylamino, dialkylamino, arylamino, diarylamino, formylamino, alkylcarbonylamino, arylcarbonylamino, heterocyclylcarbonylamino, alkoxycarbonylamino, alkoxyimino, alkylsulfonylamino, arylsulfonylamino, formyl, alkylcarbonyl, arylcarbonyl, cycloalkylcarbonyl, heterocyclylcarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl, arylthio and alkylthio.

In this respect, with the term halogen atom we intend a fluorine, chlorine, bromine or iodine atom.

With the term alkenyl or alkynyl we intend any of the aforementioned straight or branched C_2-C_6 alkyl groups further bearing a double or triple bond. Non limiting examples of alkenyl or alkynyl groups of the invention are, for instance, vinyl, allyl, 1-propenyl, isopropenyl, 1-butenyl, 2-butenyl, 3-butenyl, 2-pentenyl, 1-hexenyl, ethynyl, 2-propynyl, 4-pentynyl, and the like.

With the term perfluorinated alkyl or alkoxy we intend any of the above straight or branched C_1-C_6 alkyl or alkoxy groups which are substituted by more than one fluorine atom such as, for instance, trifluoromethyl, trifluoroethyl, 1,1,1,3,3,3-hexafluoropropyl, trifluoromethoxy and the like.

With the term alkoxy, aryloxy, heterocyclyloxy and derivatives thereof we intend any of the above alkyl, aryl or heterocyclyl groups linked to the rest of the molecule through a oxygen atom (-O-).

From all of the above, it is clear to the skilled person that any group which name is a composite name such as, for instance, cycloalkylalkyl, arylalkyl, heterocyclylalkyl, alkoxy, alkylthio, aryloxy, arylalkyloxy, alkylcarbonyloxy, arylalkyl, heterocyclylalkyl and the like, have to be intended as conventionally construed by the parts from which they derive. As an example, a group such as heterocyclylalkyloxy is an alkoxy group, e.g. alkyloxy, wherein the alkyl moiety is further substituted by a heterocyclyl group, and wherein alkyl and heterocyclyl are as above defined.

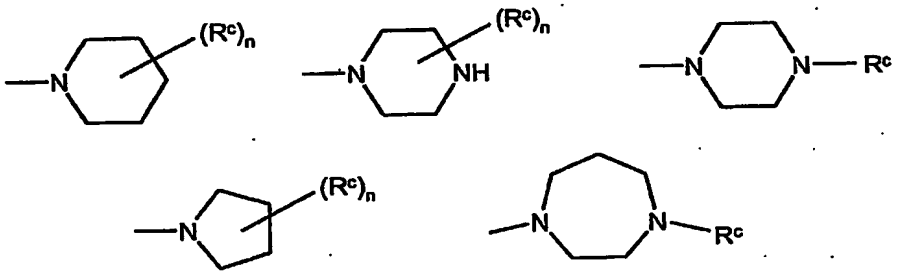
Pharmaceutically acceptable salts of the compounds of formula (I) include the acid addition salts with inorganic or organic acids, e.g., nitric, hydrochloric, hydrobromic, sulfuric, perchloric, phosphoric, acetic, trifluoroacetic, propionic, glycolic, lactic, oxalic, malonic, malic, maleic, tartaric, citric, benzoic, cinnamic, mandelic, methanesulphonic, isethionic and salicylic acid, as well as the salts with inorganic or organic bases, e.g., alkali or alkaline-earth metals, especially sodium, potassium, calcium or magnesium hydroxides, carbonates or bicarbonates, acyclic or cyclic amines, preferably methylamine, ethylamine, diethylamine, triethylamine, piperidine and the like.

A class of preferred compounds of formula (Ia) or (Ib) is represented by the derivatives wherein R is a group -COR^a, R^a is as above defined and R_1 is tert-butyl.

Another class of preferred compounds of formula (Ia) or (Ib) is represented by the derivatives wherein R is a group -$CONHR^a$, R^a is as above defined and R_1 is tert-butyl.

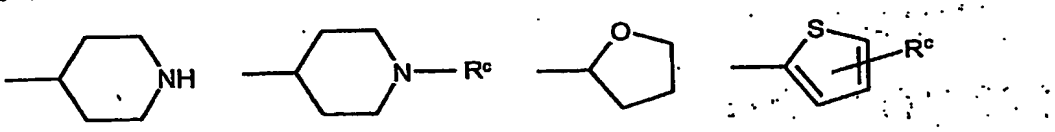
Another class of preferred compounds of formula (Ia) or (Ib) is represented by the derivatives wherein R is a group -$CONR^aR^b$, R^a and R^b are as above defined and R_1 is tert-butyl.

Another class of preferred compounds of formula (Ia) or (Ib) is represented by the derivatives wherein R is as above defined and R_1 is a group of formula (II) selected from:



wherein n and R^c have the above reported meanings.

Another class of preferred compounds of formula (Ia) or (Ib) is represented by the derivatives wherein R is as above defined and R_1 is a group of formula (II) selected from:



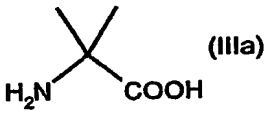
wherein R^c has the above reported meanings.

For a general reference to any specific compound of formula (I) of the invention, optionally in the form of a pharmaceutically acceptable salt, see the experimental section.

As formerly indicated, a further object of the present invention is represented by the process for preparing the compounds of formula (I).

Therefore, the compounds of formula (I) and the pharmaceutically acceptable salts may be obtained by a process comprising:

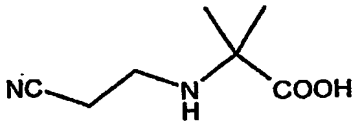
a) reacting a compound of formula (IIIa) or (IIIb)



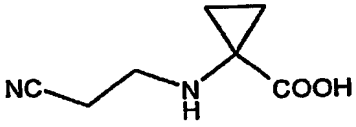
(IIIa)

(IIIb)

with acrylonitrile so as to obtain the corresponding derivative of formula (IVa) or (IVb)

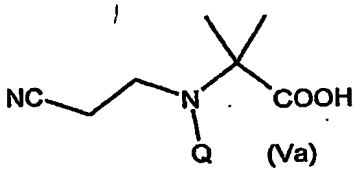


(IVa)

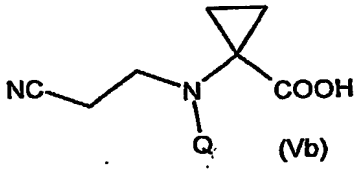


(IVb)

b) protecting the amino group of the compound of formula (IVa) or (IVb) so as to obtain the corresponding derivative of formula (Va) or (Vb)



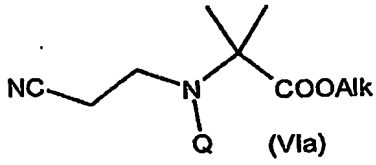
(Va)



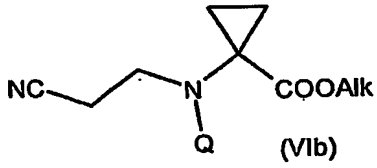
(Vb)

wherein Q is a suitable amino protecting group;

c) reacting the compound of formula (Va) or (Vb) with a suitable alkylating agent so as to obtain the corresponding ester derivative of formula (VIa) or (VIb)



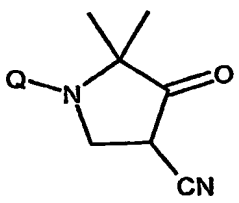
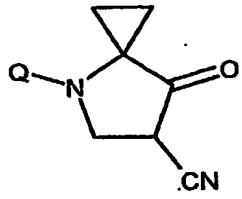
(VIa)



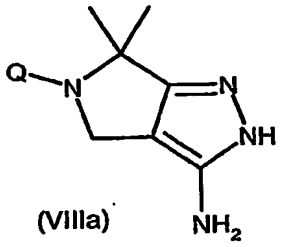
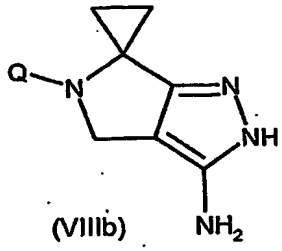
(VIb)

wherein Alk stands for a suitable C_1-C_4 alkyl group;

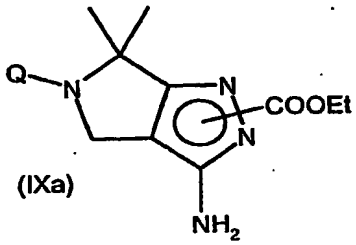
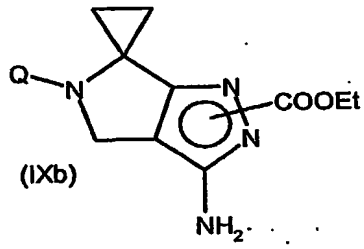
d) reacting the compound of formula (VIa) or (VIb) with sodium hydride (NaH) so as to obtain the corresponding derivative of formula (VIIa) or (VIIb)


(VIIa)

(VIIb)

e) reacting the compound of formula (VIIa) or (VIIb) with hydrazine hydrate so as to obtain the compound of formula (VIIIa) or (VIIIb)


(VIIIa)

(VIIIb)

f) reacting the compound of formula (VIIIa) or (VIIIb) with ethyl chloroformate so as to obtain the derivative of formula (IXa) or (IXb), each one in any of the two regioisomeric forms

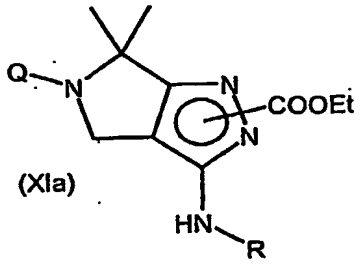

(IXa)

(IXb)

and reacting the compounds of formula (IXa) or (IXb) according to any one of the alternative steps (g.1), (g.2) or (g.3)

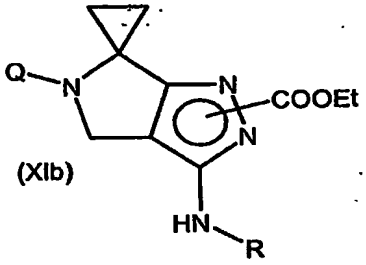
g.1) with a compound of formula (X)

$$R^aCO\text{-}Z \quad (X)$$

wherein R^a is as above defined and Z is a halogen atom, so as to obtain the compound of formula (XIa) or (XIb)



(XIa)



(XIb)

wherein R is a group -COR^a;

g.2) with a compound of formula (XII)

$$R^a\text{-}NCO \quad (XII)$$

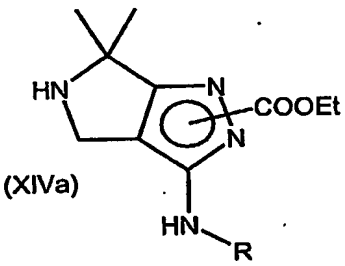
wherein R^a is as above defined so as to obtain the compound of the formula (XIa) or (XIb) wherein R is a group -$CONHR^a$; or

g.3) with a suitable amine of formula (XIII) in the presence of triphosgene or of a suitable chloroformate

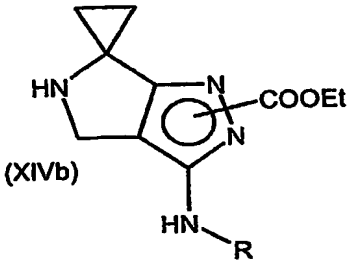
$$HNR^aR^b \quad (XIII)$$

wherein R^a and R^b are as above defined, so as to obtain the compound of formula (XIa) or (XIb) wherein R is a group -$CONR^aR^b$;

h) deprotecting the amino group of the compound of formula (XIa) or (XIb) prepared according to any one of steps from (g.1) to (g.3), so as to obtain the corresponding derivative of formula (XIVa) or (XIVb)



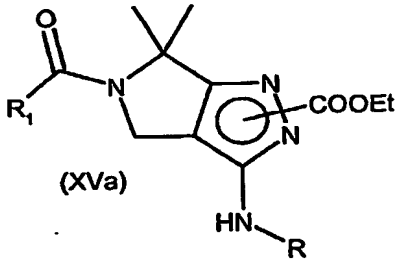
(XIVa)



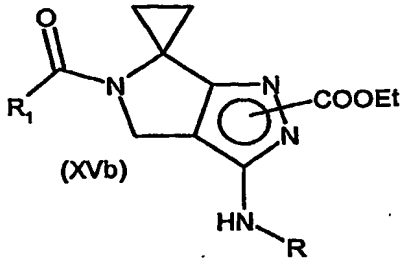
(XIVb)

wherein R has the above reported meanings; and reacting the compound of formula (XIVa) or (XIVb) according to any one of the alternative steps (i.1), (i.2) or (i.3)

i.1) with a pivaloyl halide so as to obtain a compound of formula (XVa) or (XVb)



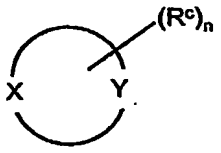
(XVa)



(XVb)

wherein R_1 is tert-butyl and R is as above defined;

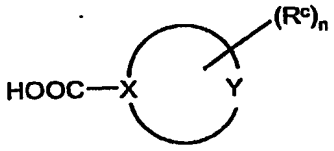
i.2) with a 5 to 7 membered heterocyclic compound of formula (XVI) in the presence of triphosgene



(XVI)

wherein X is NH and Y, R^c and n have the above reported meanings, so as to obtain the corresponding compound of formula (XVa) or (XVb) wherein R_1 is a group of formula (II), X is nitrogen and R, Y, R^c and n are as above defined;

i.3) with a 5 to 7 membered heterocyclic compound of formula (XVII) in the presence of a suitable condensing agent



(XVII)

wherein X is CH, Y is a carbon, nitrogen, oxygen or sulfur atom or it is an NH group, and Rc and n are as above defined, so as to obtain the corresponding compound of formula (XVa) or (XVb) wherein R_1 is a group of formula (II), X is CH, Y is a carbon, nitrogen, oxygen or sulfur atom or it is an NH group and R, R^c and n are as above defined;

j) reacting the compound of formula (XVa) or (XVb) prepared according to any one of steps (i.1), (i.2) or (i.3) under basic conditions, so as to obtain the corresponding derivative of formula (Ia) or (Ib) wherein R and R_1 are as above defined; and, optionally,

k) converting them into a corresponding pharmaceutically acceptable salt thereof.

The above process is an analogy process which can be carried out according to well known methods known in the art.

From all of the above, it is clear to the person skilled in the art that if a compound of formula (Ia) or (Ib), prepared according to the above process, is obtained as a mixture of isomers, their separation into the single isomers of formula (Ia) or (Ib), carried out according to conventional techniques, is still within the scope of the present invention.

Likewise, the conversion into the free compound (Ia) or (Ib) of a corresponding salt thereof, according to well-known methods, is still within the scope of the invention.

According to step (a) of the process, a compound of formula (IIIa) or (IIIb) is reacted with acrylonitrile in the presence of a suitable base, for instance sodium hydroxide. The reaction is preferably carried out in water at a temperature ranging from about –10°C to room temperature.

According to step (b) of the process, the amino group of the compound of formula (IVa) or (IVb) is protected according to conventional methods, for instance with tert-butoxycarbonyl anhydride and in the presence of a suitable solvent such as acetonitrile or dichloromethane, so as to get the corresponding derivative of formula (Va) or (Vb) wherein the amino protecting group Q just represents tert-butoxycarbonyl (boc).

According to step (c) of the process, the carboxy group of the compound of formula (Va) or (Vb) is converted into the corresponding alkyl ester derivative, for instance by operating in the presence of a suitable alkyl halide, for instance methyl iodide.

The reaction is carried out in the presence of a suitable solvent such as dimethylformamide and under basic conditions, e.g. by using sodium or potassium hydrogencarbonate.

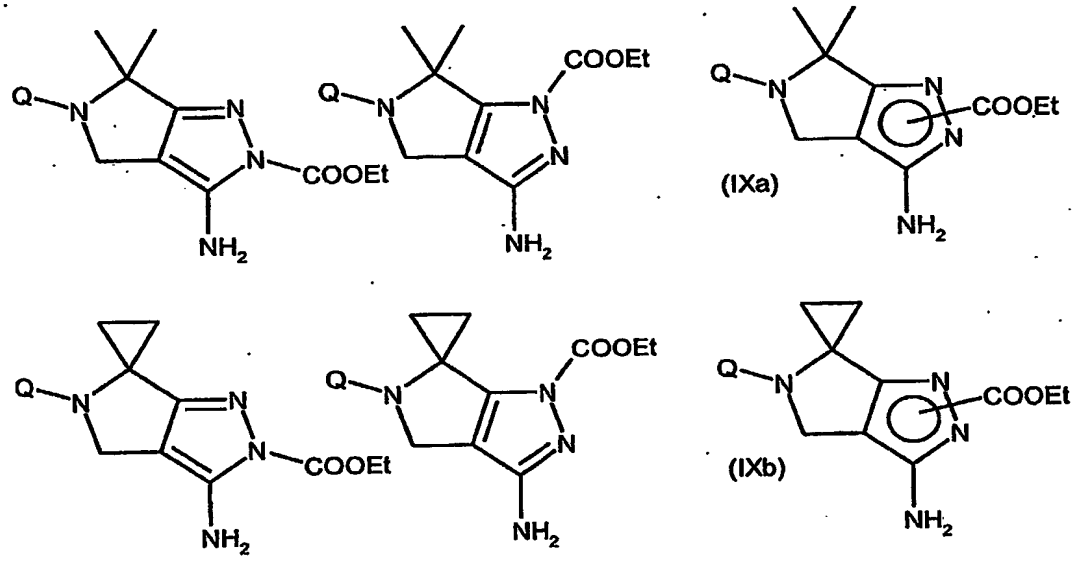
According to step (d) of the process, the compound of formula (VIa) or (VIb) is converted into the corresponding cyclic derivative of formula (VIIa) or (VIIb) through reaction with sodium hydride. The reaction is carried out in the presence of a suitable solvent such as dioxane or tetrahydrofuran at refluxing temperature.

According to step (e) of the process, the compound of formula (VIIa) or (VIIb) is reacted with hydrazine hydrate, preferably with an excess of hydrazine monohydrated, for instance up to 10 equivalents, in the presence of a suitable solvent such as halogenated hydrocarbons, lower alcohols or admixtures thereof. The reaction is preferably carried out in the presence of ethanol, by adding hydrazine to a solution of the compound of formula (VIIa) or (VIIb) and under stirring for a suitable time, e.g. for about 48 hours, at the temperature

ranging from about 20°C to about 70°C. Preferably, the above reaction is carried out also in the presence of glacial acetic acid.

According to step (f) of the process, the compound of formula (VIIIa) or (VIIIb) is reacted with ethyl chloroformate so as to get the corresponding derivative of formula (IXa) or (IXb). The reaction is carried out according to well-known operative conditions, in the presence of a suitable base, e.g. diisopropylethylamine, and of a suitable solvent such as tetrahydrofuran.

Clearly, the ethoxycarbonyl group may be bound to any one of the pyrazole nitrogen atoms of both compounds of formula (VIIIa) and (VIIIb) so as to give rise to the following regioisomers of formula (IXa) or (IXb)



In this respect, each couple of regioisomers of formula (IXa) or (IXb) may be conveniently separated according to well-known methods, for instance under chromatographic conditions, and each regioisomer so isolated subsequently worked out. In the alternative, the mixture of regioisomers can be treated as such in the subsequent steps of the process, without providing any separation.

In fact, as the ethoxycarbonyl group leading to two distinct regioisomers is finally removed at the end of the process, it is clear to the skilled person that both the above pathways can be carried out for preparing the compounds of formula (Ia) or (Ib) of the invention.

Preferably, however, the process is carried out by first separating and isolating the regioisomers of formula (IXa) or (IXb) from their mixture, as reported in the working examples, and by subsequently reacting them to the desired compounds.

According to step (g.1) of the process, the compound of formula (IXa) or (IXb) is reacted with a suitable acyl halide derivative of formula (X) wherein Z represents a halogen atom, preferably chlorine or bromine.

Typically, the compound of formula (IXa) or (IXb) is dissolved in a suitable solvent such as dichloromethane, dimethylformamide, tetrahydrofuran, dioxane or the like, and a suitable base such as triethylamine, diisopropylethylamine, sodium carbonate or the like is added. The compound of formula (X) is then added and the mixture stirred for a time of about 2 to about 15 hours, at a temperature ranging from about 20°C to about 80°C.

A suitable catalyst such as dimethylamino-pyridine may be optionally used.

According to step (g.2) of the process, the compound of formula (IXa) or (IXb) is reacted with an isocyanate derivative of formula (XII), by operating substantially as set forth in step (g.1) of the process, except that the base may not be required.

According to step (g.3) of the process, the compound of formula (IXa) or (IXb) is reacted with an amine of formula (XIII) in the presence of triphosgene or of a suitable chloroformate, for instance 4-nitrophenyl chloroformate, to get the corresponding ureido derivative. The reaction is carried out in a suitable halogenated hydrocarbon, preferably dichloromethane, or in THF, and in the presence of a suitable amine such as diisopropylethylamine or triethylamine at a temperature ranging from -70°C to room temperature.

According to step (h) of the process, the protected amino group in formula (XIa) or (XIb) is deprotected under well-known operative conditions, for instance under acidic conditions, e.g. in the presence of trifluoroacetic or hydrochloric acid.

The compound of formula (XIa) or (XIb) is thus suspended in a suitable solvent such as dichloromethane or dioxane, and treated with a concentrated solution of the selected acid. Alternatively, commercially available solutions of gaseous hydrogen chloride dissolved in dioxane (4 M HCl) may be advantageously employed. The mixture is then stirred for a time of about 2 hours to about 15 hours at a temperature ranging from about 20°C to about 40°C.

According to any one of steps (i.1), (i.2) or (i.3) of the process, the compound of formula (XIVa) or (XIVb) is further condensed with a suitable derivative so as to obtain the corresponding carboxamido or ureido derivative of formula (XVa) or (XVb).

Step (i.1) is carried out with a pivaloyl halide, preferably chloride, in a suitable solvent such as dichloromethane and under basic conditions, for instance in the presence of a suitable amine, e.g. diisopropylethylamine.

Step (i.2) is carried out with a cyclic amino derivative of formula (XVI), in the presence of triphosgene, substantially as described under step (g.3) of the process.

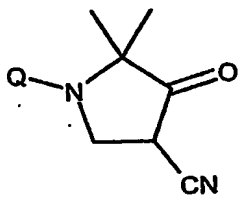
Likewise, the condensation of step (i.3) is carried out with a heterocyclic derivative of formula (XVII) bearing a carboxy group, in the presence of a suitable condensing agent such as, for instance, dicyclohexylcarbodiimide (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDC) or O-benzotriazolyl tetramethylisouronium tetrafluoroborate (TBTU), and by operating according to well-known methods for preparing carboxamido derivatives.

According to step (j) of the process, the compound of formula (XVa) or (XVb) is reacted with a suitable base, for instance triethylamine, and in the presence of a suitable solvent such as methanol or ethanol so as to obtain the desired compound of formula (Ia) or (Ib).

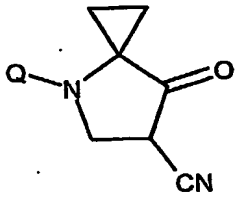
According to any variant of the process for preparing the compounds of formula (I), the starting material and any other reactant is known or easily prepared according to known methods.

As an example, whilst the starting materials of formula (IIIa) or (IIIb) are commercially available, the compounds of formula (X), (XII) and (XIII) are known or can be easily prepared according to known methods.

The intermediate compounds of formula (VIIa) or (VIIb) of the invention



(VIIa)



(VIIb)

wherein Q represents a suitable nitrogen protecting group, for instance tert-butoxycarbonyl (boc), are novel and, hence, respresent a further object of the invention.

As it will be readily appreciated, if the compounds of formula (I) prepared according to the process described above are obtained as an admixture of isomers, their separation into the single isomers of formula (I), according to conventional techniques, is within the scope of the present invention. Conventional techniques for racemate resolution include, for instance, partitioned crystallization of diastereoisomeric salt derivatives or preparative chiral HPLC.

In addition, it is clear from the above that a given compound of formula (Ia), or (Ib), may be prepared by starting from the mixture of the regioisomers of formula (IXa), or (IXb), or from each one of the two regioisomers themselves.

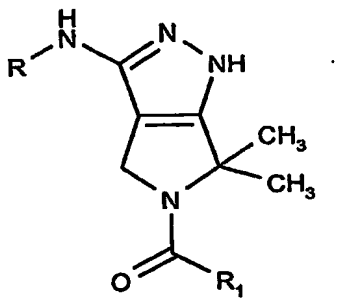
When preparing the compounds of formula (I) according to any one of the aforementioned process variants, optional functional groups within the starting materials or the intermediates thereof and which could give rise to unwanted side reactions, need to be properly protected according to conventional techniques. Likewise, the conversion of these latter into the free deprotected compounds may be carried out according to known procedures.

In addition, the compounds of formula (I) of the invention may be also prepared according to combinatorial chemistry techniques widely known in the art, by accomplishing the aforementioned reactions between the several intermediates in a serial manner and by working under solid-phase-synthesis (SPS) conditions.

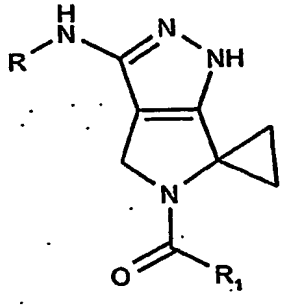
As an example, the compounds of formula (XIa) or (XIb) which are prepared according to any one of steps (g.1), (g.2) or (g.3) can be supported on a suitable polymeric resin according to operative conditions well-known in the art. More particularly, the ethoxycarbonyl group in formula (XIa) or (XIb) may be removed under basic conditions, for instance in the presence of triethylamine or diisopropylamine, and the resulting 1H-pyrazole compound further anchored to the above supporting resin, through the pyrazole nitrogen atom itself.

The supported intermediate thus obtained may be then reacted according to step (h) and any one of steps (i.1), (i.2) or (i.3) of the process, so as to obtain the corresponding compound of formula (Ia) or (Ib) of the invention still supported on the polymeric resin. Subsequent resin cleavage, for instance under basic or acidic conditions, allows to obtain the desired compounds of formula (Ia) or (Ib).

Accordingly, it is a further object of the present invention a library of two or more compounds of formula (Ia) or of two or more compounds of formula (Ib)



(Ia)

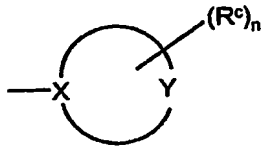


(Ib)

wherein

R is a group -COR^a, -$CONHR^a$ or -$CONR^aR^b$ wherein R^a and R^b are, each independently, hydrogen or an optionally substituted group selected from straight or branched C_1-C_6 alkyl, C_3-C_6 cycloalkyl, aryl, arylalkyl, heterocyclyl or heterocyclylalkyl or; together with the nitrogen atom to which they are bonded, R^a and R^b may form an optionally substituted 5 or 6 membered heterocycle optionally containing one additional heteroatom or heteroatomic group selected among N, NH, O or S;

R_1 is tert-butyl or a group of formula (II)



(II)

representing a 5 to 7 membered heterocyclic ring wherein X, directly linked to the rest of the molecule, represents a carbon or nitrogen atom; Y is a carbon, nitrogen, oxygen or sulfur atom or it is an NH group,, provided that at least one of X and Y is other than a carbon atom; R^c is, independently from each other and in any one of the free positions of the heterocyclic ring of formula (II), an optionally substituted group selected from straight or branched C_1-C_6 alkyl, C_3-C_6 cycloalkyl, aryl, arylalkyl, heterocyclyl, heterocyclylalkyl, amino, aminocarbonyl, carboxy, oxo (=O), alkoxycarbonyl, alkylcarbonyl or arylcarbonyl; and n is 0 or an integer from 1 to 4; or a pharmaceutically acceptable salt thereof.

PHARMACOLOGY

The compounds of formula (I) are active as protein kinase inhibitors and are therefore useful, for instance, to restrict the unregulated proliferation of tumor cells.

In therapy, they may be used in the treatment of various tumors, such as those formerly reported, as well as in the treatment of other cell proliferative disorders such as psoriasis, vascular smooth cell proliferation associated with atherosclerosis and post-surgical stenosis and restenosis and in the treatment of Alzheimer's disease.

The inhibiting activity of putative Cdk/Cyclin inhibitors and the potency of selected compounds was determined through a method of assay based on the use of the SPA technology (Amersham Pharmacia Biotech).

The assay consists of the transfer of radioactivity labelled phosphate moiety by the kinase to a biotinylated substrate. The resulting 33P-labelled biotinylated product is allowed

to bind to streptavidin-coated SPA beads (biotin capacity 130 pmol/mg), and light emitted was measured in a scintillation counter.

Inhibition assay of Cdk2/Cyclin A activity

Kinase reaction: 4 μM in house biotinylated histone H1 (Sigma # H-5505) substrate, 10 μM ATP (0.1 microCi $P^{33}\gamma$-ATP), 4.2 ng Cdk2/Cyclin A complex, inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 30 min at r.t. incubation, reaction was stopped by 100 μl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 μM ATP, containing 1 mg SPA beads. Then a volume of 110 μl is transferred to Optiplate.

After 20 min. incubation for substrate capture, 100 μl 5M CsCl were added to allow statification of beads to the top of the plate and let stand 4 hours before radioactivity counting in the Top-Count instrument

IC50 determination: inhibitors were tested at different concentrations ranging from 0.0015 to 10 μM. Experimental data were analyzed by the computer program GraphPad Prizm using the four parameter logistic equation:

y = bottom+(top-bottom)/(1+10^((logIC50-x)*slope))

where x is the logarithm of the inhibitor concentration, y is the response; y starts at bottom and goes to top with a sigmoid shape.

Ki calculation:

Experimental method: Reaction was carried out in buffer (10 mM Tris, pH 7.5, 10 mM $MgCl_2$, 0.2 mg/ml BSA, 7.5 mM DTT) containing 3.7 nM enzyme, histone and ATP (constant ratio of cold/labeled ATP 1/3000). Reaction was stopped with EDTA and the substrate captured on phosphomembrane (Multiscreen 96 well plates from Millipore). After extensive washing, the multiscreen plates are read on a top counter. Control (time zero) for each ATP and histone concentrations was measured.

Experimental design: Reaction velocities are measured at different four ATP, substrate (histone) and inhibitor concentrations. An 80-point concentration matrix was designed around the respective ATP and substrate Km values, and the inhibitor IC50 values (0.3, 1, 3, 9 fold the Km or IC50 values). A preliminary time course experiment in the absence of inhibitor and at the different ATP and substrate concentrations allow the selection

of a single endpoint time (10 min) in the linear range of the reaction for the Ki determination experiment.

Kinetic parameter estimates: Kinetic parameters were estimated by simultaneous nonlinear least-square regression using [Eq.1] (competitive inhibitor respect to ATP, random mechanism) using the complete data set (80 points):

$$v = \frac{Vm \bullet A \bullet B}{\alpha \bullet Ka \bullet Kb + \alpha \bullet Ka \bullet B + a \bullet Kb \bullet A + A \bullet B + \alpha \bullet \frac{Ka}{Ki} \bullet I \bullet (Kb + \frac{B}{\beta})} \quad \text{[Eq.1]}$$

where A=[ATP], B=[Substrate], I=[inhibitor], Vm= maximum velocity, Ka, Kb, Ki the dissociation constants of ATP, substrate and inhibitor respectively. α and β the cooperativity factor between substrate and ATP binding and substrate and inhibitor binding respectively.

In addition the selected compounds have been characterized on a panel of ser/threo kinases strictly related to cell cycle (Cdk2/Cyclin E, Cdk1/cyclin B1, Cdk5/p25, Cdk4/Cyclin D1), and also for specificity on MAPK, PKA, EGFR, IGF1-R, Aurora-2 and Akt.

Inhibition assay of Cdk2/Cyclin E activity

Kinase reaction: 10 μM in house biotinylated histone H1 (Sigma # H-5505) substrate, 30 μM ATP (0.3 microCi $P^{33}\gamma$-ATP), 4 ng GST-Cdk2/Cyclin E complex, inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 60 min at r.t. incubation, reaction was stopped by 100 μl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 μM ATP, containing 1 mg SPA beads. Then a volume of 110 μl is transferred to Optiplate.
After 20 min. incubation for substrate capture, 100 μl 5M CsCl were added to allow statification of beads to the top of the plate and let stand 4 hours before radioactivity counting in the Top-Count instrument

IC50 determination: see above

Inhibition assay of Cdk1/Cyclin B1 activity

Kinase reaction: 4 μM in house biotinylated histone H1 (Sigma # H-5505) substrate, 20 μM ATP (0.2 microCi $P^{33}\gamma$-ATP), 3 ng Cdk1/Cyclin B complex, inhibitor in a final volume of 30 μl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 20 min at r.t. incubation, reaction was

stopped by 100 µl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 µM ATP, containing 1 mg SPA beads. Then a volume of 110 µl is transferred to Optiplate.

After 20 min. incubation for substrate capture, 100µl 5M CsCl were added to allow statification of beads to the top of the Optiplate and let stand 4 hours before radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of Cdk5/p25 activity

The inhibition assay of Cdk5/p25 activity was performed according to the following protocol.

Kinase reaction: 10 µM biotinylated histone H1 (Sigma # H-5505) substrate, 30 µM ATP (0.3 microCi $P^{33}\gamma$-ATP), 15 ng CDK5/p25 complex, inhibitor in a final volume of 30 µl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 30 min at r.t. incubation, reaction was stopped by 100 µl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 µM ATP, containing 1 mg SPA beads. Then a volume of 110 µl is transferred to Optiplate.

After 20 min. incubation for substrate capture, 100µl 5M CsCl were added to allow statification of beads to the top of the plate and let stand 4 hours before radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of Cdk4/Cyclin D1 activity

Kinase reaction: 0,4 uM µM mouse GST-Rb (769-921) (# sc-4112 from Santa Cruz) substrate, 10 µM ATP (0.5 µCi $P^{33}\gamma$-ATP), 100 ng of baculovirus expressed GST-Cdk4/Cyclin D1, suitable concentrations of inhibitor in a final volume of 50 µl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, 7.5 mM DTT+ 0.2mg/ml BSA) were added to each well of a 96 U bottom well plate. After 40 min at 37 °C incubation, reaction was stopped by 20 µl EDTA 120 mM.

Capture: 60 µl were transferred from each well to MultiScreen plate, to allow substrate binding to phosphocellulose filter. Plates were then washed 3 times with 150 µl/well PBS Ca^{++}/Mg^{++} free and filtered by MultiScreen filtration system.

Detection: filters were allowed to dry at 37°C, then 100 µl/well scintillant were added and ^{33}P labeled Rb fragment was detected by radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of MAPK activity

Kinase reaction: 10 µM in house biotinylated MBP (Sigma # M-1891) substrate, 15 µM ATP (0.15 microCi $P^{33}\gamma$-ATP), 30 ng GST-MAPK (Upstate Biothecnology # 14-173), inhibitor in a final volume of 30 µl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 30 min at r.t. incubation, reaction was stopped by 100 µl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 µM ATP, containing 1 mg SPA beads. Then a volume of 110 µl is transferred to Optiplate.

After 20 min. incubation for substrate capture, 100µl 5M CsCl were added to allow statification of beads to the top of the Optiplate and let stand 4 hours before radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of PKA activity

Kinase reaction: 10 µM in house biotinylated histone H1 (Sigma # H-5505) substrate, 10 µM ATP (0.2 microM $P^{33}\gamma$-ATP), 0.45 U PKA (Sigma # 2645), inhibitor in a final volume of 30 µl buffer (TRIS HCl 10 mM pH 7.5, $MgCl_2$ 10 mM, DTT 7.5 mM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 90 min at r.t. incubation, reaction was stopped by 100 µl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 µM ATP, containing 1 mg SPA beads. Then a volume of 110 µl is transferred to Optiplate.

After 20 min. incubation for substrate capture, 100 µl 5M CsCl were added to allow statification of beads to the top of the Optiplate and let stand 4 hours before radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of EGFR activity

Kinase reaction: 10 µM in house biotinylated MBP (Sigma # M-1891) substrate, 2 µM ATP (0.04 microCi $P^{33}\gamma$-ATP), 36 ng insect cell expressed GST-EGFR, inhibitor in a final volume of 30 µl buffer (Hepes 50 mM pH 7.5, $MgCl_2$ 3 mM, $MnCl_2$ 3 mM, DTT 1 mM,

$NaVO_3$ 3μM + 0.2 mg/ml BSA) were added to each well of a 96 U bottom. After 20 min at r.t. incubation, reaction was stopped by 100 μl PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 μM ATP, containing 1 mg SPA beads. Then a volume of 110 μl is transferred to Optiplate.

After 20 min. incubation for substrate capture, 100μl 5M CsCl were added to allow statification of beads to the top of the Optiplate and let stand 4 hours before radioactivity counting in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of IGF1-R activity

The inhibition assay of IGF1-R activity was performed according to the following protocol.

Kinase reaction: 10 μM biotinylated MBP (Sigma cat. # M-1891) substrate, 0-20 μM inhibitor, 6 μM ATP, 1 microCi ^{33}P-ATP, and 22.5 ng GST-IGF1-R (pre-incubated for 30 min at room temperature with cold 60 μM cold ATP) in a final volume of 30 μl buffer (50 mM HEPES pH 7.9, 3 mM $MnCl_2$, 1 mM DTT, 3 μM $NaVO_3$) were added to each well of a 96 U bottom well plate. After incubation for 35 min at room temperature, the reaction was stopped by addition of 100 μl PBS buffer containing 32 mM EDTA, 500 μM cold ATP, 0.1% Triton X100 and 10mg/ml streptavidin coated SPA beads. After 20 min incubation, 110 μL of suspension were withdrawn and transferred into 96-well OPTIPLATEs containing 100 μl of 5M CsCl. After 4 hours, the plates were read for 2 min in a Packard TOP-Count radioactivity reader.

Inhibition assay of Aurora-2 activity

Kinase reaction: 8 μM biotinylated peptide (4 repeats of LRRWSLG), 10 μM ATP (0.5 uCi $P^{33}\gamma$-ATP), 15 ng Aurora2, inhibitor in a final volume of 30 μl buffer (HEPES 50 mM pH 7.0, $MgCl_2$ 10 mM, 1 mM DTT, 0.2 mg/ml BSA, 3μM orthovanadate) were added to each well of a 96 U bottom well plate. After 30 minutes at room temperature incubation, reaction was stopped and biotinylated peptide captured by adding 100 μl of bead suspension.

Stratification: 100 μl of CsCl2 5 M were added to each well and let stand 4 hour before radioactivity was counted in the Top-Count instrument.

IC50 determination: see above

Inhibition assay of Cdc7/dbf4 activity

The inhibition assay of Cdc7/dbf4 activity was performed according to the following protocol.

The Biotin-MCM2 substrate is trans-phosphorylated by the Cdc7/Dbf4 complex in the presence of ATP traced with γ^{33}-ATP. The phosphorylated Biotin-MCM2 substrate is then captured by Streptavidin-coated SPA beads and the extent of phosphorylation evaluated by β counting.

The inhibition assay of Cdc7/dbf4 activity was performed in 96 wells plate according to the following protocol.

To each well of the plate were added:

- 10 µl substrate (biotinylated MCM2, 6 µM final concentration)
- 10 µl enzyme (Cdc7/Dbf4, 12.5 nM final concentration)
- 10 µl test compound (12 increasing concentrations in the nM to µM range to generate a dose-response curve)
- 10 µl of a mixture of cold ATP (10µM final concentration) and radioactive ATP (1/2500 molar ratio with cold ATP) was then used to start the reaction which was allowed to take place at 37°C.

Substrate, enzyme and ATP were diluted in 50 mM HEPES pH 7.9 containing 15 mM $MgCl_2$, 2 mM DTT, 3 µM $NaVO_3$, 2mM glycerophosphate and 0.2mg/ml BSA. The solvent for test compounds also contained 10% DMSO.

After incubation for 20 minutes, the reaction was stopped by adding to each well 100 µl of PBS pH 7.4 containing 50 mM EDTA, 1 mM cold ATP, 0.1% Triton X100 and 10 mg/ml streptavidin coated SPA beads.

After 15 minutes of incubation at room temperature to allow the biotinylated MCM2-streptavidin SPA beads interaction to occur, beads were trapped in a 96 wells filter plate (Unifilter^R GF/B^{TM}) using a Packard Cell Harvester (Filtermate), washed with distilled water and then counted using a Top Count (Packard).

Counts were blank-subtracted and then the experimental data (each point in triplicate) were analyzed for IC50 determination using a non-linear regression analysis (Sigma Plot).

Given the above inhibition assays, the compounds of formula (I) of the invention resulted to possess a remarkable cdk inhibitory activity. See, as an example, the following

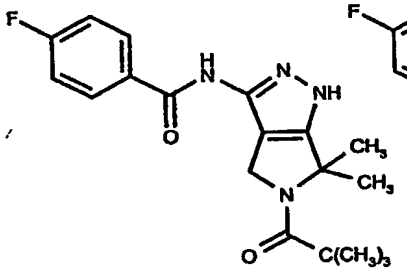
experimental data (IC_{50}) of two representative compounds of the invention of formula (Ia) and (Ib) being tested against Cdk2/Cyclin A:

Compound 1: N-[5-(2,2-dimethylpropanoyl)-6,6-dimethyl-1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-yl]-4-fluorobenzamide (IC_{50} 0.030 μM); and

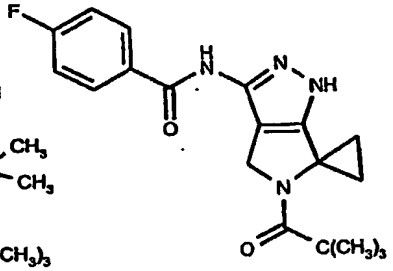
Compound 2: N-[5-(2,2-dimethylpropanoyl)-1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-6-spirocyclopropan-3-yl]-4-fluorobenzamide (IC_{50} 0.025 μM).

Surprisingly, the said inhibitory activity resulted to be markedly superior that that of a very close compound of the prior art WO 02/12242, herewith referred to as Reference compound (see compound 1143, bottom of page 76; and example 19, compound bridging pages 242-3 of WO 02/12242), used for comparative purposes and tested against Cdk2/Cyclin A, as formerly reported:

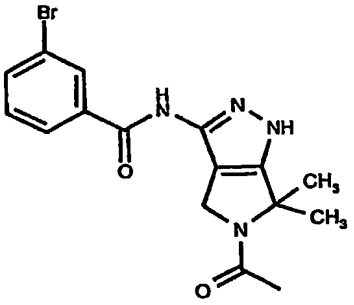
Reference Compound: N-[5-acetyl-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazol-3-yl]-(3-bromo)benzamide (IC_{50} 1.7 μM)



Compound 1



Compound 2



Reference compound

So far, the novel compounds of the invention are unexpectedly endowed with a cdk inhibitory activity significantly higher than that of the structurally closest prior art compounds of WO 02/12242 and are thus particularly advantageous, in therapy, against proliferative disorders associated with an altered cell cycle dependent kinase activity.

The compounds of the present invention can be administered either as single agents or, alternatively, in combination with known anticancer treatments such as radiation therapy or chemotherapy regimen in combination with cytostatic or cytotoxic agents, antibiotic-type

agents, alkylating agents, antimetabolite agents, hormonal agents, immunological agents, interferon-type agents, cyclooxygenase inhibitors (e.g. COX-2 inhibitors), matrixmetalloprotease inhibitors, telomerase inhibitors, tyrosine kinase inhibitors, anti-growth factor receptor agents, anti-HER agents, anti-EGFR agents, anti-angiogenesis agents (e.g. angiogenesis inhibitors), farnesyl transferase inhibitors, ras-raf signal transduction pathway inhibitors, cell cycle inhibitors, other cdks inhibitors, tubulin binding agents, topoisomerase I inhibitors, topoisomerase II inhibitors, and the like.

As an example, the compounds of the invention can be administered in combination with one or more chemotherapeutic agents such as, for instance, exemestane, formestane, anastrozole, letrozole, fadrozole, taxane and derivatives such as paclitaxel or docetaxel, encapsulated taxanes, CPT-11, camptothecin derivatives, anthracycline glycosides, e.g., doxorubicin, idarubicin, epirubicin, etoposide, navelbine, vinblastine, carboplatin, cisplatin, estramustine phosphate, celecoxib, tamoxifen, raloxifen, Sugen SU-5416, Sugen SU-6668, Herceptin, and the like, optionally within liposomal formulations thereof.

If formulated as a fixed dose, such combination products employ the compounds of this invention within the dosage range described below and the other pharmaceutically active agent within the approved dosage range.

Compounds of formula (I) may be used sequentially with known anticancer agents when a combination formulation is inappropriate.

The compounds of formula (I) of the present invention, suitable for administration to a mammal, e.g., to humans, can be administered by the usual routes and the dosage level depends upon the age, weight, conditions of the patient and administration route.

For example, a suitable dosage adopted for oral administration of a compound of formula (I) may range from about 10 to about 500 mg per dose, from 1 to 5 times daily. The compounds of the invention can be administered in a variety of dosage forms, e.g., orally, in the form tablets, capsules, sugar or film coated tablets, liquid solutions or suspensions; rectally in the form suppositories; parenterally, e.g., intramuscularly, or through intravenous and/or intrathecal and/or intraspinal injection or infusion.

The present invention also includes pharmaceutical compositions comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable excipient, which may be a carrier or a diluent.

The pharmaceutical compositions containing the compounds of the invention are usually prepared following conventional methods and are administered in a suitable pharmaceutical form. For example, the solid oral forms may contain, together with the active

compound, diluents, e.g., lactose, dextrose saccharose, sucrose, cellulose, corn starch, or potato starch; lubricants, e.g., silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents, e.g., starches, arabic gum, gelatine methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disintegrating agents, e.g., starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. These pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film-coating processes.

The liquid dispersions for oral administration may be, e.g., syrups, emulsions and suspensions. As an example, the syrups may contain, as carrier, saccharose or saccharose with glycerine and/or mannitol and sorbitol.

The suspensions and the emulsions may contain, as examples of carriers, natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspension or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g., sterile water, olive oil, ethyl oleate, glycols, e.g., propylene glycol and, if desired, a suitable amount of lidocaine hydrochloride.

The solutions for intravenous injections or infusions may contain, as a carrier, sterile water or preferably they may be in the form of sterile, aqueous, isotonic, saline solutions or they may contain propylene glycol as a carrier.

The suppositories may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g., cocoa butter, polyethylene glycol, a polyoxyethylene sorbitan fatty acid ester surfactant or lecithin.

With the aim of better illustrating the present invention, without posing any limitation to it, the following examples are now given.

General methods

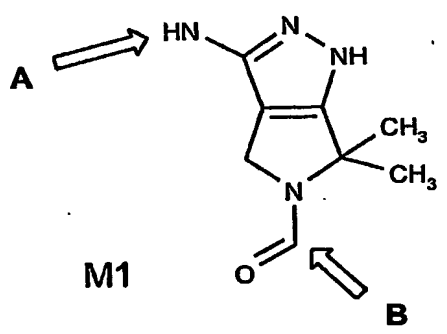
Before taking into consideration the synthetic preparation of the specific compounds of formula (I) of the invention, for instance as reported in the following examples, attention should be given to the fact that some compounds are herewith listed and indicated according to their chemical name whilst others, most of them, have been conveniently and unambiguously identified through a coding system, together with their ^{1}H-NMR data (see following tables III, IV and V).

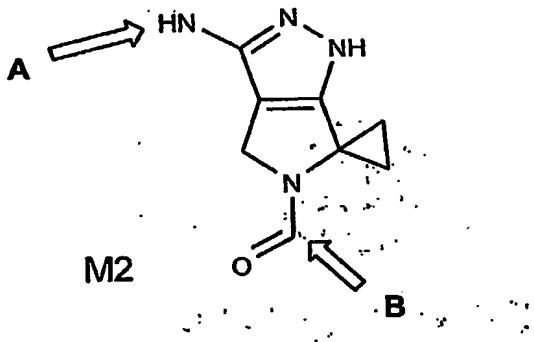
Each code, in particular, identifies a single specific compound of formula (Ia) or (Ib) and consists of three units A-M-B.

A represents any substituent R [see formula (Ia) or (Ib)] and is attached to the rest of the molecule through the -NH-group; each specific A group is represented and consecutively numbered in the following table I.

Likewise, B represents any substituent R_1 [see formula (Ia) or (Ib)] and is attached to the rest of the molecule through the carbonyl (CO) group; each specific B group is represented and consecutively numbered in the following table II.

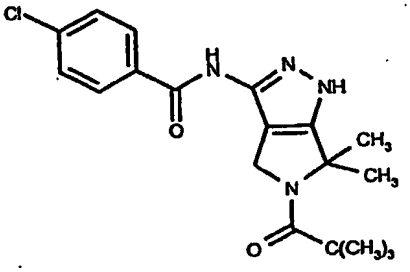
M refers to the central core of the divalent moiety which is substituted by groups A and B; in particular, M may vary from M1 or M2 as per the formulae below, each identifying the central core of a compound having formula (Ia) or (Ib), respectively:



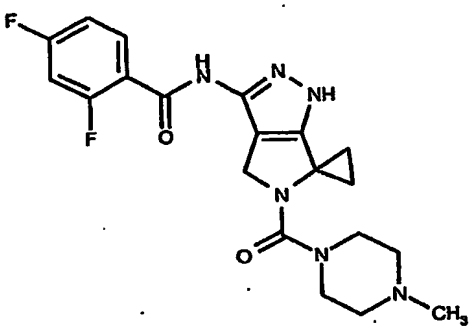


For ease of reference, all of the A and B groups of tables I and II have been identified with the proper chemical formula also indicating the point of attachment with the rest of the molecule M.

Therefore, just as an example, the compound A06-M1-B01 of table III represents the compound of formula (Ia) having the central M1 core, being substituted by the group A06 and by the group B01, in the positions indicated by the arrows; likewise, the compound A04-M2-B08 of table V represents the compound of formula (Ib) having the central M2 core, being substituted by the group A04 and by the group B08, in the positions indicated by the arrows:



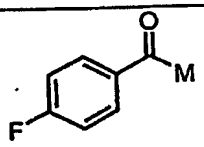
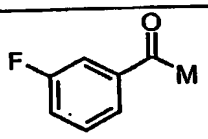
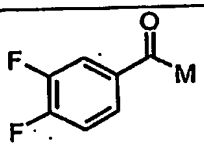
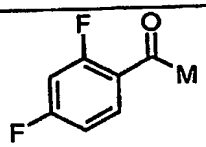
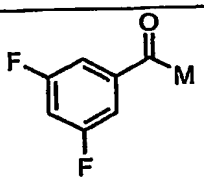
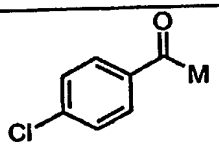
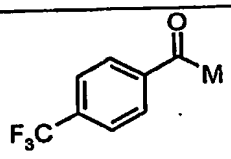
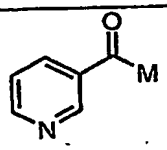
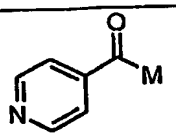
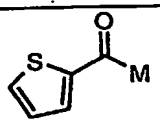
A06-M1-B01

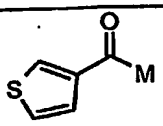
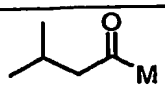
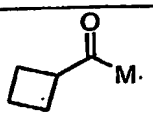
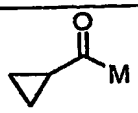
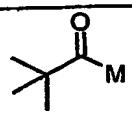
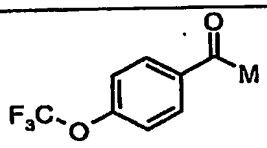
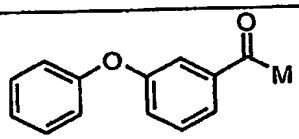
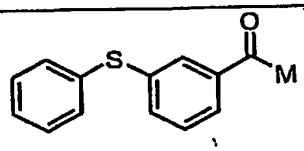
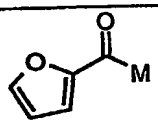
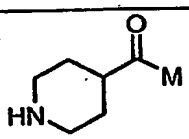
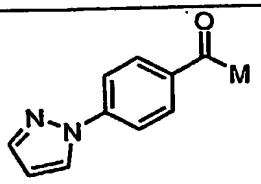
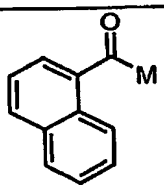
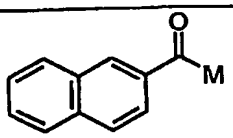
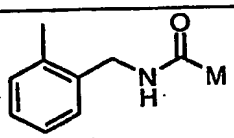
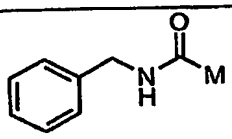
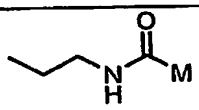
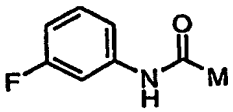
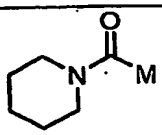


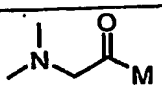
A04-M2-B08

From the above, it is clear to the skilled person that this coding system allows to easily and unambiguously identify the compounds of formula (Ia) and (Ib) of the invention, as per tables III, IV and V, together with their analytical ^{1}H-NMR data.

Table I

A01		A02	
A03		A04	
A05		A06	
A07		A08	
A09		A10	

A11		A12	
A13		A14	
A15		A16	
A17		A18	
A19		A20	
A21		A22	
A23		A24	
A25		A26	
A27		A28	

A29			

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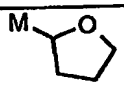
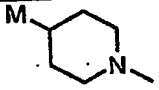
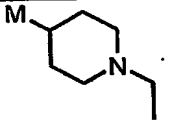
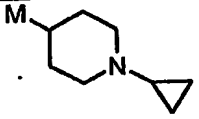
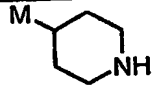
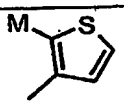
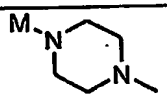
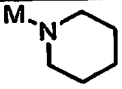
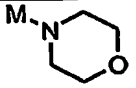
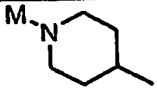
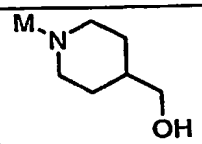
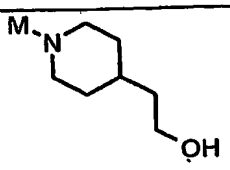
B01		B02	
B03		B04	
B05		B06	
B07		B08	
B09		B10	
B11		B12	
B13			

Example 1

N-(2-cyanoethyl)-2-methylalanine

50 g (0.48 mol) of 2-methylalanine were added to a cooled solution (water/ice) of NaOH (19.6 g) in water (100 ml). Once the solution had turned clear, 34 ml (0.5 mol) of acrylonitrile were dropped on cooling. The mixture was left overnight. After 18 hours, 28 ml of acetic acid were added on cooling (water/ice); a white solid precipitated; 200 ml of 95% ethanol were dropped in the flask, stirring was continued for 1 hour, then the mixture was allowed to stand in a fridge for 2-3 hours. After filtration, the solid was collected and dried in an oven at 80°C. The filtrates were evaporated and taken up with ethanol (160 ml). On cooling a further amount of product was obtained, which was filtered and dried. 72 g were obtained from the first filtration. Total yield claimed: 95%.

ESI MS: m/z 157 (MH+)

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47 (s, 1H), 2.70 (t, 2H, J = 6.22 Hz), 2.57 (t, 2H, J = 6.22 Hz), 1.18 (s, 6H).

By working in an analogous manner the following compound was prepared:

1-[(2-Cyanoethyl)amino]cyclopropanecarboxylic acid

EI MS: m/z 154 (M), 136 (M-H_2O), 114 (M-CH2CN), 68 (100%, cyclopr=C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47 (s, 1H), 2.86 (t, 2H, J = 6.6 Hz), 2.48 (t, 2H, J = 6.6 Hz), 1.09 (dd, 2H, J = 6.9 Hz, J = 4.1 Hz), 0.86 (dd, 2H., J = 6.9 Hz, J = 4.1 Hz).

Example 2

N-(tert-butoxycarbonyl)-N-(2-cyanoethyl)-2-methylalanine

44.5 g (0.285 mol) of N-(2-cyanoethyl)-2-methylalanine and 51.7 g of tetramethylammonium hydroxide pentahydrate were dissolved in acetonitrile (2 l) at 40° C and when a clear solution was obtained , 112 g of Boc_2O were added. The mixture was left for 24 hours at 40° C. The day after, a further 20 g of Boc_2O were added while maintaining 40°C. Every 8-12 hours a 20 g of Boc_2O were added up to a total of 192 g. After 4 days the solvent was evaporated, the residue taken up with water (1000 ml), washed twice with ethyl ether (500 ml). The aqueous fraction was brought to pH 3-4 with citric acid and extracted with ethyl acetate, washed with water (200 ml) and concentrated. 52 g of title compound were obtained. (Claimed yield: 72%).

ESI MS: m/z 237 (M-H-)

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.18 (s, 1H), 3.52 (t, 2H, J = 6.8 Hz), 2.67 (t, 2H, J = 6.8 Hz), 1.38 (s, 6H), 1.36 (s, 9H).

By working in an analogous manner the following compound was prepared:

***tert*-Butyl 6-cyano-7-oxo-4-azaspiro[2.4]heptane-4-carboxylate**

ESI MS: m/z 235 (M-H-)

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (s, 1H), 3.43 (m, 2H), 2.71 (m, 2H), 1.38 (m, 13H).

Example 3

Methyl N-(*tert*-butoxycarbonyl)-N-(2-cyanoethyl)-2-methylalaninate

62 g (0.23 mol) of N-(tert-butoxycarbonyl)-N-(2-cyanoethyl)-2-methylalanine were dissolved in 350 ml of DMF and 50 g of KHCO3 were added. Few minutes after, 30 ml of MeI were dropped and the mixture was stirred at room temperature for 6 hours. Then a further 15 ml of MeI were added. The mixture was left at room temperature overnight. After dilution with 1.5 l of water, the solution was extracted with ethyl acetate (3 times). The organic phases were washed with a small amount of water, dried over sodium sulfate, evaporated and dried at the mech. Pump. 60.5 g (97%) of methyl N-(*tert*-butoxycarbonyl)-N-(2-cyanoethyl)-2-methylalaninate were obtained.

ESI MS: m/z 288 (M+NH4);

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.55 (m, 5H), 2.70 (t, 2H, J = 6.7 Hz)), 1.40 (s, 6H), 1.36 (s, 9H).

By working in an analogous manner the following compound was prepared:

Methyl 1-[(*tert*-butoxycarbonyl)(2-cyanoethyl)amino]cyclopropanecarboxylate

ESI MS: m/z 286 (M+NH4);

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.61 (s, 3H), 3.42 (t, 2H, J = 6.7 Hz), 2.71 (m, 2H), 1.62-1.07 (m, 13H).

Example 4

***tert*-Butyl 4-cyano-3-hydroxy-2,2-dimethyl-2,5-dihydro-1H-pyrrole-1-carboxylate**

45 g of methyl N-(*tert*-butoxycarbonyl)-N-(2-cyanoethyl)-2-methylalaninate were dissolved in dioxane (240 ml) under nitrogen and 7.9 g of sodium hydride were added. The mixture was refluxed for 6 hours, and then left to stand overnight at room temperature. (TLC:

CH2Cl2/EtOH 90:10). The solvent was evaporated, water was added (1000 ml) and the mixture was brought to pH 3-4 with citric acid. The aqueous layer was extracted 4 times with ethyl acetate, the extracts washed with a limited amount of water, evaporated. Then the residue was taken up with hexane, evaporated and crystallized from hexane. 33.1 g of *tert*-butyl 4-cyano-3-hydroxy-2,2-dimethyl-2,5-dihydro-1H-pyrrole-1-carboxylate were obtained (Claimed yield: 85%).

ESI MS: m/z 237 (M-H-)

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H), 3.97 (2s, 2H, conformers), 1.48 (s, 6H), 1.47 (s, 9H).

By working in an analogous manner the following compound was prepared:

***tert*-Butyl 6-cyano-7-oxo-4-azaspiro[2.4]heptane-4-carboxylate**

ESI MS: m/z 235 (M-H-)

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.63 (t, 1H, J = 9.8 Hz), 4.24 (t, 1H, J = 10.2 Hz), 3.74 (t, 1H, J = 10.2 Hz), 2.05-1.67 (m, 2H), 1.37 (s, 9H), 1.18-0.95 (m, 2H).

Example 5

***tert*-Butyl 3-amino-6,6-dimethyl-2,6-dihydropyrrolo[3,4-c]pyrazole-5(4H)-carboxylate**

32 g of *tert*-Butyl 4-cyano-3-hydroxy-2,2-dimethyl-2,5-dihydro-1H-pyrrole-1-carboxylate (0.134 mol) were added to 430 ml of abs. ethanol. To this solution, 9 ml (0.18 mol) of hydrazine hydrate are added, followed by 12 ml of glac. AcOH (1.5 eq); The mixture was stirred at 60 °C for 48 hours, the ethanol was removed, the residue was taken up with 400 ml of saturated sodium hydrogen carbonate aqueous, then the water was extracted several times with ethyl acetate up to total extraction of the desired product. The organic phases were dried and evaporated. After purification by flash chromatography (eluent: CHCl3-EtOH 97:3) and trituration with a mixture of hexane/ethyl acetate 9/1 25g of a title compoud were obtained. Total yield 30.5 g (Claimed yield: 88%)

ESI MS: m/z 253 (MH+)

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.06-4.10 (2s, 2H, conformers), 1.48 (2s, 6H, conformers), 1.47 (2s, 9H, conformers).

By working in an analogous manner the following compound was prepared:

***tert*-Butyl-3-amino-2,6-dihydropyrrolo[3,4-c]pyrazole-6-spirocyclopropane-5(4H)-carboxylate**

ESI MS: m/z 251 (MH+)

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.12 (bs, 1H), 5.13 (bs, 2H), 4.33-4.16 (m, 2H), 1.91-1.57 (m, 2H), 1.38 (s, 9H), 0.83-0.65 (m, 2H).

Example 6

5-tert-butyl 2-ethyl 3-amino-6,6-dimethylpyrrolo[3,4-c]pyrazole-2,5(4H,6H)-dicarboxylate and 5-tert-butyl 1-ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate

15 g of *tert*-butyl 3-amino-6,6-dimethyl-2,6-dihydropyrrolo[3,4-c]pyrazole-5(4H)-carboxylate (5.94 mol) were dissolved in anhydrous THF (150 ml) and treated at 0°C, under an Ar atmosphere, first with diisopropylethylamine (50 ml) and then with $ClCO_2Et$ (4.65 ml, 1eq.) dropwise. 90 minutes later, the solvent was diluted with EtOAc (1 l), washed with water and then with brine, dried on sodium sulfate, evaporated. The crude product was purified by flash chromatography (hexane/AcOEt 20/80) to afford 7.3 g of 5-tert-butyl 2-ethyl 3-amino-6,6-dimethylpyrrolo[3,4-c]pyrazole-2,5(4H,6H)-dicarboxylate as major compound in 38% yield together with 5.7 g of and 5-tert-butyl 1-ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate 5 in 30% yield.

5-*tert*-Butyl 2-ethyl 3-amino-6,6-dimethylpyrrolo[3,4-c]pyrazole-2,5(4H,6H)-dicarboxylate

ESI MS: m/z 325 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.53 (s, 1H), 4.35 (q, 2H, J = 7.07 Hz), 4.10 (2s, 2H, conformers), 1.51-1.50 (m, 6H), 1.43-1.41 (2s, 9H, conformers), 1.29 (t, 3H, J = 7. 07 Hz).

5-tert-butyl 1-ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate

ESI MS: m/z 325 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 5.70 (s, 1H), 4.31 (q, 2H, J = 7.1 Hz) 4.18-4.13 (2s, 2H, conformers), 1.71 (2s, 6H, conformers), 1.45 (2s, 9H, conformers), 1.27 (t, 3H, J = 7.1 Hz).

By working in an analogous manner the following compounds were prepared:

5-*tert*-Butyl 2-ethyl 3-amino-pyrrolo[3,4-c]pyrazole-6-spirocyclopropane-2,5(4H,6H)-dicarboxylate

Rf: 0.6 (7:3 dichloromethane/ethyl acetate)

ESI MS: m/z 323 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 6.55 (s, 1H), 4.30 (q, 2H, J = 7.1 Hz), 4.27 (bs, 2H), 1.93 (m, 2H), 1.39 (s, 9H) 1.28 (t, 3H, J = 7.1 Hz), 0.86 (m, 2H).

5-*tert*-butyl 1-ethyl 3-amino-4,6-dihydropyrrolo[3,4-c]pyrazole-6-spirocyclopropane-1,5-dicarboxylate

Rf: 0.25 (7:3 dichloromethane/ethyl acetate)

ESI MS: m/z 323 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.26 (bs, 2H), 4.21 (q, 2H, J = 7.0 Hz), 1.85 (m, 4H), 1.38 (s, 9H), 1.23 (t, 3H, J = 7.0 Hz).

Example 7

5-tert-butyl 1-ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-4,6-dihydro pyrrolo[3,4-c]pyrazole-1,5-dicarboxylate

5-tert-Butyl 1-ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate (2.0 g, 6.16 mmol) was dissolved in THF (40 ml), treated first with diisopropylethylamine (5.4 ml, 30.8 mmol) and then, at 0°C, 4-fluorobenzoyl chloride (800 µl, 6.77 mmol) dissolved in THF (8 ml) was added dropwise. The reaction mixture was stirred at room temperature for 5 hours, concentrated and dissolved in methylene chloride, washed with saturated sodium hydrogen carbonate aqueous solution and with brine. The organic phase was dried over sodium sulfate, evaporated and purified by flash chromatography (eluent: Hexane/ EtOAc 80/20) to afford 2.50 g of title compound in 90% yield.

ESI MS: m/z 447 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47 (s, 1H), 8.04-8.17 (m, 2H), 7.25-7.37 (m, 2H), 4.44-4.47 (2s, 2H, conformers), 4.43 (q, 2H, J = 7.1 Hz), 1.73-1.75 (2s, 6H, conformers), 1.43-1.46 (2s, 9H, conformers), 1.33 (t, 3H, J = 7.1 Hz).

By working in an analogous manner the following compounds were prepared.

5-*tert*-Butyl 2-ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethylpyrrolo[3,4-c]pyrazole-2,5(4H,6H)-dicarboxylate

ESI MS: m/z 447 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.78 (s, 1H), 7.99-7.95 (m, 2H), 7.47-7.40 (m, 2H), 4.51-4.49 (2s, 2H, conformers), 4.43 (q, 2H, J = 7.1 Hz), 1.60-1.59 (2s, 6H), 1.46-1.43 (2s, 9H, conformers), 1.34 (t, 3H, J = 7.1 Hz).

5-*tert*-Butyl 2-ethyl 3-[(4-fluorobenzoyl)amino]-pyrrolo[3,4-c]pyrazole-6-spirocyclopropane-2,5(4H,6H)-dicarboxylate

ESI MS: m/z 445 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.81 (s, 1H), 8.06-7.95 (m, 2H), 7.49-7.39 (m, 2H), 4.67 (bs, 2H), 4.41 (q, 2H, J = 7.1 Hz), 2.10-1.80 (m, 2H), 1.41 (s, 9H), 1.32 (t, 3H, J = 7.1 Hz), 1.06-0.93 (m, 2H).

Example 8

5-tert-butyl 1-ethyl 3-({[(3-fluorophenyl)amino]carbonyl}amino)-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate

5-*tert*-Butyl 1-ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate (3.0 g, 9.24 mmol) was dissolved in anhydrous THF (50 ml), treated at room temperature with 3-fluorophenyl-isocyanate (1.4 g, 10.21 mmol, 1.1 eq) and stirred overnight. The following day the reaction mixture was evaporated, taken up with methylene chloride and washed with brine. The organic phase was dried over sodium sulfate and evaporated to dryness. Purification by flash chromatography (CH_2Cl_2/EtOAc 90/10) afforded 3.05 g (yield 71%) of title compound

ESI MS: m/z 462 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.74 (s, 1H), 9.05 (s, 1H), 7.44 (m, 1H), 7.33 (m, 1H), 7.16 (m, 1H), 6.84 (m, 1H), 4.43 (m, 4H), 1.76 (2s, 6H), 1.48 (2s, 9H, conformers), 1.36 (t, 3H, J = 7.1 Hz).

Example 9

5-*tert*-Butyl 1-ethyl 3-[(piperidine-1-carbonyl)-amino]-6,6-dimethyl-4,6-dihydro pyrrolo[3,4-c]pyrazole-1,5-dicarboxylate

To a solution of triphosgene (550 mg, 1.85 mmol, 0.4 eq) in THF (50 ml) was added, at -40°C, a solution of 5-*tert*-butyl 1-ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate (1.5 g, 4.62 mmol) and diisopropylethylamine (1.8 ml, 2.2 eq) in THF (50 ml)). After 3 hours, a solution of piperidine (690 µl, 1.5 eq) and diisopropylethylamine (1.2 µl, 1.5 eq) in THF (25 ml) was added. The reaction was allowed to reach room temperature in 2 hours (TLC: EtOAc/hexane 90/10). After evaporation of the solvent the solid was dissolved in methylene chloride and the solution was washed with brine, the organic phase was dried over sodium sulphate and concentrated. The solid was

purified by flash chromatography (eluent: EtOAc/hexane 50/50). The solid was treated with diisopropylether and filtered to afford 1.45 g of title compound in 72% yield.

ESI MS: m/z 436 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.36 (s, 1H), 4.46 (m, 4H), 3.40 (m, 4H), 1.76 (2s, 6H), 1.54 (m, 6H), 1.44 (2s, 9H, conformers), 1.36 (t, 3H, J = 7.1 Hz).

Example 10

Ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c] pyrazole-1(4H)-carboxylate hydrochloride

5-*tert*-Butyl 1-ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-1,5-dicarboxylate (2.5, 5.59 mmol) was dissolved in dioxane (50 ml) and treated with HCl 4M in dioxane (28 ml, 20 eq). After 2 hours at 40°C (TLC: CH_2Cl_2/MeOH 90/10) the reaction mixture was concentrated and the residue was treated with diethyl ether, filtered to afford the title compound (2.09 g) as solid in 98% yield.

ESI MS: m/z 347 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H), 8.06-8.11 (m, 2H), 7.28-7.34 (m, 2H), 4.40 (q, 2H, J = 7.1 Hz), 3.92 (s, 2H), 1.42 (s, 6H), 1.33 (t, 3H, J = 7.1 Hz).

By working in an analogous manner the following compounds were prepared:

Ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c] pyrazole-2(4H)-carboxylate hydrochloride

ESI MS: m/z 347 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.92 (s, 1H), 9.89 (s, 2H), 8.02 (m, 2H), 7.49 (m, 2H), 4.61 (s, 2H), 4.51 (q, 2H, J = 7.1 Hz), 1.69 (s, 6H), 1.39 (t, 3H, J = 7.1 Hz).

Ethyl 3-[(4-fluorobenzoyl)amino]-5,6-dihydropyrrolo[3,4-c]pyrazole-6-spirocyclopropane-2(4H)-carboxylate hydrochloride

ESI MS: m/z 345 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (bs, 1H), 10.00 (bs, 2H), 7.93-8.04 (m, 2H), 7.39-7.53 (m, 2H), 4.69 (bs, 2H), 4.41 (q, 2H, J = 7.1 Hz), 1.68 (dd, 2H, J = 8.6 Hz, J = 6.1 Hz), 1.41 (dd, 2H, J = 8.6 Hz, J = 6.1 Hz), 1.33 (t, 3H, J = 7.1 Hz).

Ethyl 3-({[(3-fluorophenyl)amino]carbonyl}amino)-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c]pyrazole-1(4H)-carboxylate hydrochloride

ESI MS: m/z 362 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.97 (s, 2H), 9.35 (s, 2H), 7.48 (m, 1H), 7.34 (m, 1H), 7.16 (m, 1H), 6.85 (m, 1H), 4.47 (s, 2H), 4.43 (q, 2H, J = 7.1 Hz), 1.77 (s, 6H), 1.37 (t, 3H, J = 7.1 Hz).

Example 11

Ethyl 5-(2,2-dimethylpropanoyl)-3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c]pyrazole-1(4H)-carboxylate

Ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c]pyrazole-1(4H)-carboxylate hydrochloride (2.0 g, 5.77 mmol), in dichloromethane (70 ml), was treated with diisopropylethylamine (3.0 ml, 17.3 mmol, 3 eq) and at 0°C with pivaloyl chloride (780 µL, 6.3 mmol, 1.1 eq). Gradually the reaction was brought to room temperature and stirred overnight (TLC: CH_2Cl_2/EtOAc 90/10). The solution was washed with saturated sodium hydrogen carbonate aqueous solution and brine. The organic phase was dried over sodium sulphate, evaporated and purified by flash chromatography (eluent: CH_2Cl_2/ EtOAc 90/10) to afford 2.03 g of title compound in 82% yield.

ESI MS: m/z 431 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.51 (s, 1H), 8.05-8.14 (m, 2H), 7.23-7.37 (m, 2H), 4.90 (s, 2H), 4.42 (q, 2H, J = 7.1 Hz), 1.80 (s, 6H), 1.33 (t, 3H, J = 7.1 Hz), 1.22 (s, 9H).

By working in an analogous manner the following compound was prepared:

Ethyl 5-(2,2-dimethylpropanoyl)-3-[(4-fluorobenzoyl)amino]-5,6-dihydro pyrrolo[3,4-c]pyrazole-6-spirocyclopropane-2(4H)-carboxylate

ESI MS: m/z 429 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.81 (bs, 1H), 7.96-8.04 (m, 2H), 7.38-7.48 (m, 2H), 5.10 (bs, 2H), 4.42 (q, 2H, J = 7.1 Hz), 2.33 (dd, 2H, J = 6.8 Hz, J = 4.2 Hz), 1.32 (t, 3H, J = 7.1 Hz), 1.22 (s, 9H), 0.90 (dd, 2H, J = 6.8 Hz, J = 4.2 Hz).

Example 12

N-[5-(2,2-dimethylpropanoyl)-6,6-dimethyl-1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-yl]-4-fluorobenzamide

Ethyl 5-(2,2-dimethylpropanoyl)-3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c]pyrazole-1(4H)-carboxylate (2.0 g, 4.64 mmol) was dissolved in methanol (60 ml), treated with TEA (6.45 ml, 46.4 mmol, 10 eq) and stirred overnight at

room temperature (TLC: CH_2Cl_2/MeOH 95/5). After evaporation, the solid was treated with diethyl ether/hexane and filtered to afford 1.43 g of title compound in 86% yield.

ESI MS: m/z 359 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 (bs, 1H), 10.91 (bs, 1H), 7.98-8.11 (m, 2H), 7.20-7.44 (m, 2H), 4.66-4.92 (bs, 2H), 1.64 (s, 6H), 1.21 (s, 9H).

By working in an analogous manner the following compounds were prepared:

N-[5-(2,2-Dimethylpropanoyl)-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-6-spirocyclopropan-3-yl]-4-fluorobenzamide

ESI MS: m/z 357 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.59-12.47 (bs, 1H), 10.94 (bs, 1H), 8.02-8.11 (m, 2H), 7.27-7.37 (m, 2H), 4.99 (s, 2H), 2.25 (dd, 2H, J = 6.5 Hz, J = 4.4 Hz), 1.20 (s, 9H), 0.79 (dd, 2H, J = 6.5 Hz, J = 4.4 Hz).

N-{6,6-dimethyl-5-[(2R)-tetrahydrofuran-2-ylcarbonyl]-1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-yl}-4-fluorobenzamide

ESI MS: m/z 373 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.49 (bs, 1H), 10.96 (bs, 1H), 8.09 (m, 2H), 7.34 (m, 2H), 4.86 (m, 2H), 4.56 (t, 1H, J = 6.47 Hz), 3.83 (m, 2H), 2.02 (m, 2H), 1.86 (m, 2H), 1.68 (s, 6H), 1.21 (s, 9H).

N-{6,6-dimethyl-5-[(2S)-tetrahydrofuran-2-ylcarbonyl]-1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-yl}-4-fluorobenzamide

ESI MS: m/z 373 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.49 (bs, 1H), 10.96 (bs, 1H), 8.09 (m, 2H), 7.34 (m, 2H), 4.86 (m, 2H), 4.56 (t, 1H, J = 6.47 Hz), 3.83 (m, 2H) 2.02 (m, 2H), 1.86 (m, 2H), 1.68 (s, 6H), 1.21 (s, 9H).

Additional compounds of formula (Ia) and (Ib) were also prepared, as reported in the following table III; for explanatory notes concerning the coding system identifying each specific compound, see pages 28-29.

Table III

A02M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.45 (bs, 1H), 10.99 (s, 1H), 7.84 (m, 2H), 7.52 (m, 1H), 7.40 (m, 1H), 4.86 (s, 2H), 1.65 (s, 6H), 1.21 (s, 9H).
A03M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.46 (bs, 1H), 11.01 (s, 1H), 8.05 (m, 1H), 7.88 (m, 1H), 7.54 (m, 1H), 4.86 (s, 2H), 1.65 (s, 6H), 1.21 (s, 9H).

A04M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.45 (bs, 1H), 10.89 (s, 1H), 7.7 (m, 1H), 7.38 (m, 1H), 7.20 (s, 1H), 4.89 (s, 2H), 1.68 (s, 6H), 1.24 (s, 9H).
A05M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.54 (bs, 1H), 11.07 (s, 1H), 7.76 (s, 2H), 7.51 (s, 1H), 4.89 (s, 2H), 1.68 (s, 6H), 1.25 (s, 9H).
A06M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.43 (bs, 1H), 10.98 (s, 1H), 7.98 (d, 2H, J = 8.0 Hz), 7.54 (d, 2H, J = 8.0 Hz), 4.86 (s, 2H), 1.65 (s, 6H), 1.21 (s, 9H).
A07M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.47 (bs, 1H), 11.16 (s, 1H), 8.16 (d, 2H, J = 7.9 Hz), 7.85 (d, 2H, J = 7.9 Hz), 4.88 (s, 2H), 1.65 (s, 6H), 1.21 (s, 9H).
A08M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.50 (bs, 1H), 11.17 (s, 1H), 9.13 (s, 1H,), 8.76 (m, 1H), 8.33 (m, 1H) 7.51 (m, 1H), 4.91 (s, 2H), 1.69 (s, 6H), 1.25 (s, 9H).
A09M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.54 (bs, 1H), 11.25 (s, 1H), 8.75 (d, 2H), 7.91 (d, 2H), 4.92 (s, 2H), 1.69 (s, 6H), 1.25 (s, 9H).
A10M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.47 (bs, 1H), 11.00 (s, 1H), 8.12 (m, 1H), 7.86 (m, 1H), 7.12 (m, 1H), 4.86 (s, 2H), 1.68 (s, 6H), 1.25 (s, 9H).
A11M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.11 (bs, 1H), 10.77 (s, 1H), 8.45 (s, 1H), 7.69 (m, 2H), 4.89 (s, 2H), 1.68 (s, 6H), 1.25 (s, 9H).
A12M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.22 (bs, 1H), 10.31 (s, 1H), 4.76 (s, 2H), 2.12 (m, 3H), 1.61 (s, 6H), 1.19 (s, 9H), 0.87 (d, 6H, J = 6.5 Hz).
A13M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.21 (bs, 1H), 10.19 (bs, 1H), 4.80 (s, 2H), 3.28 (m, 1H), 2.25-1.70 (m, 6H), 1.60 (s, 6H), 1.20 (s, 9H).
A14M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.35 (bs, 1H), 10.38 (bs, 1H), 4.75 (s, 2H), 3.24 (m, 1H), 2.21-1.67 (m, 4H), 1.60 (s, 6H), 1.20 (s, 9H).
A15M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.3 (bs, 1H), 9.89 (s, 1H), 4.80 (s, 2H), 1.64 (s, 6H), 1.23 (s, 9H),1.21 (s, 9H).
A16M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.35 (bs, 1H), 11.02 (s, 1H), 8.09 (d, 2H, J = 8.2 Hz), 7.47 (d, 2H, J = 8.2 Hz), 4.85 (s, 2H), 1.64 (s, 6H), 1.21 (s, 9H).
A19M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.48 (bs, 1H), 10.82 (s, 1H), 8.43 (m, 1H), 7.45 (m, 1H), 6.70 (m, 1H), 4.85 (s, 2H), 1.67 (s, 6H), 1.25 (s, 9H).
A20M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.26 (bs, 1H), 10.38 (s, 1H), 4.80 (s, 2H), 3.04 (m, 2H), 2.52 (m, 3H), 1.64 (s, 10H), 1.23 (s, 9H).
A24M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (bs, 1H), 8.87 (s, 1H), 7.19 (m, 4H), 6.91(bs, 1H), 4.73 (s, 2H), 4.32 (d, 2H, J = 5.85 Hz), 2.30 (s, 3H), 1.63 (s, 6H),

	1.22 (s, 9H).
A25M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.31-12.05 (2bs, 1H), 8.48 (s, 1H), 7.30 (m, 5H), 7.00 (bs, 1H), 4.73 (s, 2H), 4.33 (d, 2H, J = 5.85 Hz), 1.63 (s, 6H), 1.22 (s, 9H).
A28M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.22 (bs, 1H), 9.42 (s, 1H), 4.85 (s, 2H), 3.41 (m, 4H), 1.67 (s, 6H), 1.49 (m, 6H), 1.22 (s, 9H).
A29M1B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.36 (bs, 1H), 10.08 (s, 1H), 4.82 (s, 2H), 3.15 (bs, 2H), 2.32 (s, 6H), 1.65 (s, 6H), 1.23 (s, 9H).
A02M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.29 (bs, 1H), 11.05 (s, 1H), 7.9-7.35 (m, 3H), 5.03 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 (m, 2H).
A04M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.21 (bs, 1H), 10.90 (s, 1H), 7.79 (m, 1H), 7.40 (m, 1H), 7.21 (m, 1H), 5.03 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.83 (m, 2H).
A05M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.30 (bs, 1H), 11.17 (s, 1H), 7.77 (s, 2H), 7.51 (s, 1H), 5.04 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 (m, 2H).
A06M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (bs, 1H), 11.04 (s, 1H), 8.03 (d, 2H, J = 8.0 Hz), 7.61 (d, 2H, J = 8.0 Hz), 5.03 (s, 2H), 2.29 (m, 2H), 11.24 (s, 9H), 0.83 (m, 2H).
A07M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.32 (bs, 1H), 11.22 (s, 1H), 8.21 (d, 2H, J = 7.9 Hz), 7.91 (d, 2H, J = 7.9 Hz), 5.05 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 (m, 2H).
A10M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.23 (bs, 1H), 11.02 (s, 1H), 8.10 (m, 1H), 7.87 (m, 1H), 7.22 (m, 1H), 5.01 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.84 (m, 2H).
A13M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (bs, 1H), 10.28 (bs, 1H), 4.96 (s, 2H), 3.32 (m, 1H), 2.25 (m, 8H), 1.23 (s, 9H), 0.78 (m, 2H).
A14M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.03 (bs, 1H), 10.72 (bs, 1H), 4.91 (s, 2H), 2.4-0.7 (m, 18H).
A15M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (bs, 1H), 9.92 (s, 1H), 4.94 (s, 2H), 2.25 (m, 2H), 1.22 (s, 18H), 0.78 (m, 2H).
A19M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.21 (bs, 1H), 10.82 (s, 1H), 7.92 (m, 1H), 7.51 (m, 1H), 6.69 (m, 1H), 5.01 (s, 2H), 2.29 (m, 2H), 1.24 (s, 9H), 0.83 (m, 2H).
A28M2B01	^{1}H NMR (400 MHz, DMSO-d_6): δ 11.83 (bs, 1H), 9.03 (s, 1H), 4.87 (s, 2H), 3.41 (m, 4H), 2.23 (m, 2H), 1.49 (m, 6H), 1.22 (s, 9H), 0.76 (m, 2H).

A01M2B02	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.25 (bs, 1H), 10.98 (bs, 1H), 8.11 (m, 2H), 7.34 (m, 2H), 4.96 (m, 2H), 4.56 (dd, 1H, J = 7.43 Hz), 3.77 (m, 2H), 2.24 (m, 2H), 2.03 (m, 2H), 1.87 (m, 2H), 0.93 (s, 2H).

Example 13

N-{6,6-dimethyl-5-[(1-methylpiperidin-4-yl)carbonyl]-1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-3-yl}-4-fluorobenzamide

1-Methyl-piperidine-4-carboxylic acid hydrochloride (0.290 g, 1.61 mmol, 1.2 eq) in dichloromethane (25 ml) was treated with diisopropylethylamine (1.14 ml, 6.5 mmol, 5 eq) and TBTU (0.55 g, 1.7 mmol, 1.3 eq) at room temperature for 1 hour, and then ethyl 3-[(4-fluorobenzoyl)amino]-6,6-dimethyl-5,6-dihydropyrrolo[3,4-c]pyrazole-1(4H)-carboxylate hydrochloride (0.500 g, 1.3 mmol) was added. The reaction was stirred overnight (TLC: CH_2Cl_2/MeOH 90/10). The solution was washed with saturated sodium hydrogen carbonate aqueous solution and brine, the organic phase was dried over sodium sulphate and concentrated. The residue was dissolved in methanol (16 ml), treated with TEA (2 ml, 14.3 mmol, 11 eq) and stirred overnight at room temperature. (TLC: CH_2Cl_2/MeOH/NH_4OH 90/10/1). After evaporation, the solid was purified by flash chromatography (eluent: CH_2Cl_2/MeOH/NH_4OH 90/10/2). The solid was treated with diisopropylether and filtered to afford 0.36 g of title compound in 69% yield.

ESI MS: m/z 400 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.48 (bs, 1H), 10.97 (bs, 1H), 8.09 (m, 2H), 7.35 (m, 2H), 4.75 (bs, 2H), 2.87 (m, 2H), 2.40 (m, 1H), 2.24 (s, 3H), 2.05 (m, 2H), 1.67 (m, 10H).

By working in an analogous manner the following compound was prepared:

N-[5--[(1-methylpiperidin-4-yl)carbonyl]-2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazol-6-spirocyclopropan-3-yl]-4-fluorobenzamide

ESI MS: m/z 398 (MH+);

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.20 (bs, 1H), 11.00 (bs, 1H), 8.10 (m, 2H), 7.36 (m, 2H), 4.91 (s, 2H), 2.85 (m, 4H), 2.40-1.5 (m, 10H), 0.89 (m, 2H).

Additional compounds of formula (Ia) and (Ib) were also prepared, as reported in the following table IV; for explanatory notes concerning the coding system identifying each specific compound, see pages 28-29.

Table IV

A04M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.48 (bs, 1H), 10.93 (s, 1H), 7.75 (m, 2H), 7.22 (m, 2H), 4.75 (bs, 2H), 2.85 (m, 4H), 2.21 (m, 8H), 1.67 (bs, 6H).
A05M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.6 (bs, 1H), 11.19 (s, 1H), 7.72 (m, 2H), 7.51 (m, 1H), 4.80 (bs, 2H), 3.34-2.76 (m, 8H), 1.69 (m, 10H).
A06M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (bs, 1H), 11.06 (s, 1H), 8.02 (m, 2H), 7.63 (m, 1H), 4.81 (s, 2H), 3.33-2.75 (m, 8H), 1.69 (m, 10H).
A07M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.60 (bs, 1H), 11.25 (s, 1H), 8.19 (d, 2H, J = 7.9 Hz), 7.92 (d, 2H, J = 7.9 Hz), 4.83 (s, 2H), 3.33 (m, 8H), 1.69 (m, 10H).
A10M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.52 (bs, 1H), 11.07 (s, 1H), 8.12 (m, 2H), 7.86 (m, 1H), 7.22 (m, 1H), 4.79 (bs, 2H), 3.47-3.02 (m, 5H), 2.76 (m, 3H), 1.99-1.77 (m, 4H), 1.68 (bs, 6H).
A12M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.29 (bs, 1H), 10.40 (s, 1H), 4.65 (s, 2H), 2.86 (m, 2H), 2.35 (m, 1H), 2.22 (bs, 3H), 2.17 (m, 2H), 2.02 (m, 3H), 1.64 (m, 10H), 0.92 (d, 6H, J = 6.6 Hz).
A13M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.29 (bs, 1H), 10.27 (s, 1H), 4.69 (s, 2H), 3.35 (m, 1H), 2.87 (m, 2H), 2.38 (m, 1H), 2.24 (bs, 3H), 2.20 (m, 6H), 2.07 (m, 2H), 1.63 (m, 10H).
A14M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.30 (bs, 1H), 10.72 (s, 1H), 4.63 (s, 2H), 2.86 (m, 2H), 2.34 (m, 1H), 2.23 (bs, 3H), 2.04 (m, 2H), 1.83 (m, 1H), 1.63 (m, 10H), 0.79 (m, 4H).
A16M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.30 (bs, 1H), 11.10 (s, 1H), 8.13 (d, 2H, J = 7.9 Hz), 7.51 (d, 2H, J = 7.9 Hz), 4.75 (s, 2H), 2.86 (m, 4H), 2.39 (m, 1H), 2.22 (s, 3H); 1.69 (m, 10H).
A21M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (bs, 1H), 11.00 (s, 1H), 8.65 (d, 1H, J = 2.5 Hz), 8.16 (d, 2H, J = 8.3 Hz), 8.01 (d, 2H, J = 8.3 Hz), 7.83 (d, 1H, J = 1.6 Hz), 6.62 (dd, 1H, J = 2.5 Hz), 2.86 (m, 4H), 2.38 (m, 1H), 2.22 (s, 3H), 1.69 (m, 10H).
A22M1B03	^{1}H NMR (400 MHz, DMSO-d_6): δ 12.44 (bs, 1H), 11.12 (s, 1H), 8.25 (m, 1H), 8.09 (m, 1H), 8.03 (m, 1H), 7.75 (m, 1H), 7.60 (m, 3H), 4.82 (bs, 2H), 2.84 (m, 2H), 2.38 (m, 1H), 2.19 (s, 3H), 1.98 (m, 2H), 1.70 (bs, 10H).

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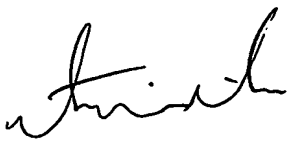
VIII-4-1 -2-1	Name:	RICKWOOD, Martin
VIII-4-1 -2-2	Residence: (city and either US State, if applicable, or country)	CLARKS GREEN, Pennsylvania
VIII-4-1 -2-3	Mailing address:	303 Rabbit Run CLARKS GREEN, PA 18411 United States of America
VIII-4-1 -2-4	Citizenship:	US
VIII-4-1 -2-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	
VIII-4-1 -2-6	Date: 5.04.2004 (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	
VIII-4-1 -3-1	Name:	MORGAN, Kimberly
VIII-4-1 -3-2	Residence: (city and either US State, if applicable, or country)	MOOSIC, Pennsylvania
VIII-4-1 -3-3	Mailing address:	720 Church Street MOOSIC, PA 18507 United States of America
VIII-4-1 -3-4	Citizenship:	US
VIII-4-1 -3-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	
VIII-4-1 -3-6	Date: 5.04.2004 (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	

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VIII-4-1 -4-1	Name:	WANIGATUNGA, Sirisoma
VIII-4-1 -4-2	Residence: (city and either US State, if applicable, or country)	LARGO, Florida
VIII-4-1 -4-3	Mailing address:	13863 Kimberly Drive LARGO, FL 33774 United States of America
VIII-4-1 -4-4	Citizenship:	US
VIII-4-1 -4-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	
VIII-4-1 -4-6	Date: (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	